Scientific Coordinator
Kélig Mahé
IREMER Institut, Fisheries laboratory, Sclerochronology centre
kelig.mahe@ifremer.fr

Software Coordinator
Sébastien Fave
Noesis
sf@noesis.fr

User Guide Coordinator
Jean Couteau
Code Lutin
couteau@codelutin.com

TNPC

User guide

TNPC 5

TNPC

User guide

TNPC : User guide

Legal Notice - License Creative Commons - By-SA

Table of Contents

List of Figures

List of Examples

Chapter 1. Generalities

1.1. Introduction

TNPC 5 [1] is a dedicated software aimed to assist CS [2] interpretation, financed by the French institute IFREMER [3]. Several years have been necessary to develop this software. The project begun in 1994 with Hervé Troadec's work, then other people contributed to it such as Ronan Fablet, Hélène de Pontual and André Ogor. TNPC provides several features that will be explained in details in this documentation :

- Manage databases of images and associated data,
- Batch processes on CS images,
- Image acquisition with automatic stage, scanners, mosaics,
- Save predefined data (radials, intensity curves) and user data (for example age interpretation).

TNPC is based on the Visilog 6™ platform. Most of the features are Visilog 6™ features and you can find the appropriate documentation in the Visilog 6™ documentation on the TNPC CD-ROM. We made the choice not to reproduce in this documentation all the Visilog 6™ manual.

Note

TNPC uses some Microsoft® Office™ features. You will need to install it on your computer to be able to use all TNPC features.

Warning

All this documentation describe the expert version of TNPC. The routine version being a simplification of it, you can also use this documentation. If you need something that is not present in the routine version, you will need to install the expert version instead.

On this documentation, graphical user interface elements are decorated to make them distinguishable. Buttons are represented like that : I am a button. Other graphical elements such as window title, tab title, important words are displayed like that : I AM A GRAPHICAL ELEMENT. They might not look exactly the same on your screen.

Some terms are specific to halieutics or sclerochronology. They are explained in the glossary at the end of this documentation. The same way, some terms are abbreviated in this documentation. They are explained on the first occurrence and in the glossary. If you do not know what something means, you might find an explanation there.

1 Traitement Numérique des Pièces Calcifiées - Numerical Treatment of Calcified Structures

2 Calcified Structures

3 IFREMER institute website : *http://www.ifremer.fr/anglais*

To help you find exactly the information you want, you might find an index at the end of this documentation. This index specifies the pages where a specific topic is explained. You might also use the table of contents.

1.2. Installing TNPC

Installing TNPC is quite easy. You just have to launch the `Setup.exe` file located on the installation CD-ROM and follow the instructions. The only choice you have to make is between the routine and expert interfaces (following the use you will make of TNPC). At the end of the installation process, you can install TNPC documentation and your camera if needed.

Note

Routine mode will only allow you to read and annotate otoliths, perform simple acquisition whereas the expert mode is the full version, allowing you to perform acquisition, automatic estimation…

Note

If you have your own version of Visilog 6™, you must install it before installing TNPC.

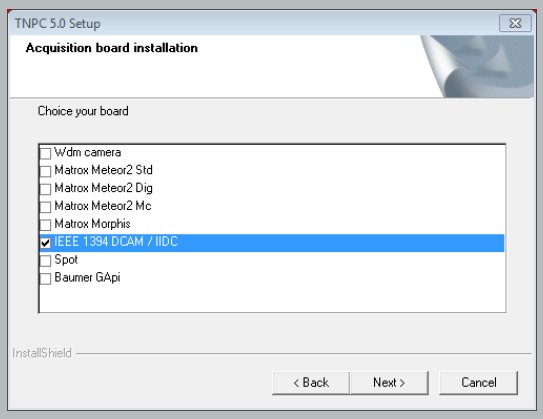
Note

If you got a camera, check that it is well mounted on the microscope. If the camera is badly mounted, you will not be able to make mosaics.

Note

When using a camera, IEE1394 drivers must be replaced by drivers delivered by Noesis. You can install them during TNPC installation by selecting Install acquisition board driver(s).

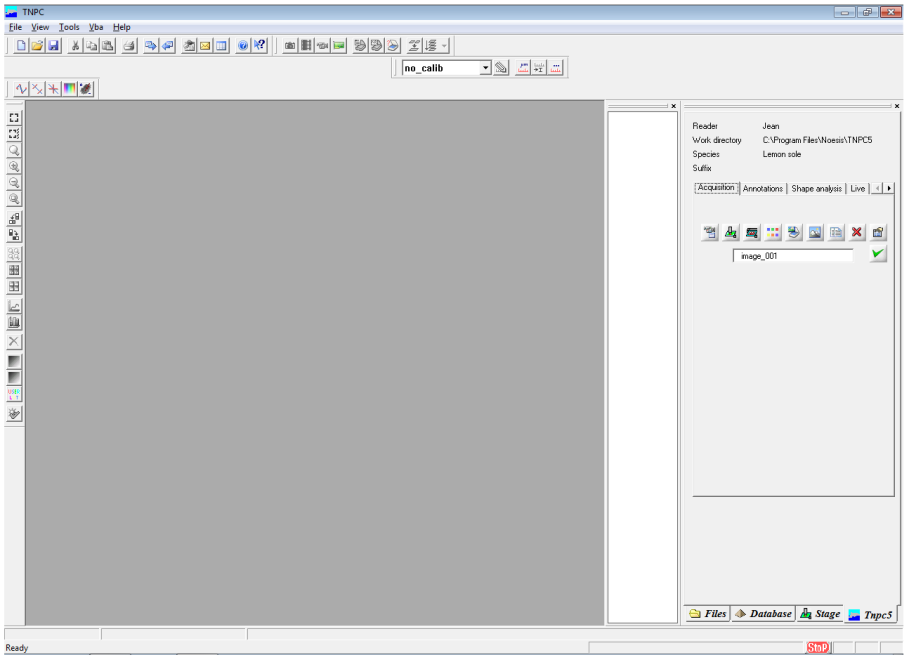
Figure 1.1. Install acquisition board drivers



1.3. TNPC user interface

As said previously, TNPC is built upon Visilog 6™ platform. Most of the user interface comes from Visilog 6™. The only TNPC user interface is located on the right of the screen, on the TNPC tab. The other tabs contain features of the Visilog 6™ platform.

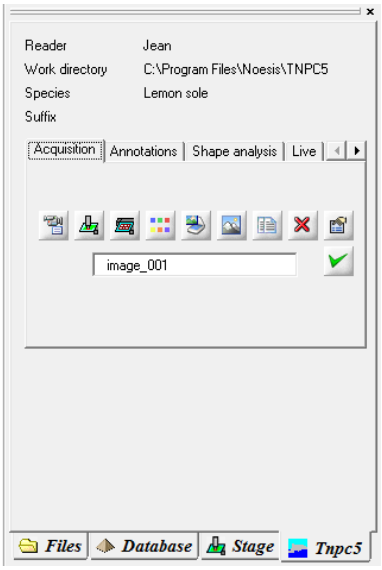
Figure 1.2. Visilog 6™ and TNPC Window



The TNPC interface is split between the global information and seven tabs :

- *Acquisition*
- *Annotations*
- *Shape analysis (Expert mode)*
- *Live (Expert mode)*
- *Apprenticeship of individual age (Expert mode)*
- *Automatic estimation of individual age (Expert mode)*
- *Automatic estimation of age structure (Expert mode)*

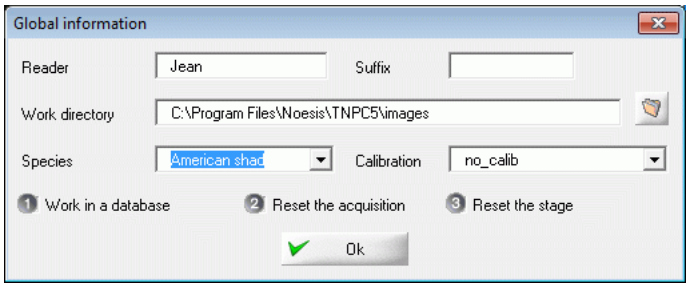
Figure 1.3. TNPC User Interface



The global information are present whatever tab is selected. It gathers general information asked at TNPC start-up such as :

- Reader : By default it is the name of the current Windows session.
- Work directory : The directory where all the files you are working on are located.
- Species : The species you are working on.
- Suffix : This suffix can be chosen by the user to distinguish the files treated by TNPC and the others. It is added to all the images generated by TNPC. This is the only point that is not mandatory.

Figure 1.4. Global information input



1. Open the database module at TNPC startup.
2. Reset the acquisition at TNPC startup. You must select this if your camera was not previously powered on.
3. Reset the communication with the motorized stage controller. You must select this if your controller was not previously powered on.

Figure 1.5. Global information

Note

If you want to change global information, you have to restart TNPC.

The ACQUISITION tab is used to acquire images from a camera and a microscope equipped with a motorised stage, or a scanner, and create mosaics. Acquisition parameters can be changed from one session to another, or from one image to another. The image can be stored in the database as well as preferences. To know how to perform acquisition, go to *Chapter 3, Image acquisition* .

The ANNOTATIONS tab is used to set and store CS interpretation (reading axis, rings positions, false rings and checks positions, annotations…). You can also compute the otolith growth and fish-length back-calculation. All the acquired information (age, CS interpretation, growth patterns…) can be stored in the image database and exported in text or Excel™ format. To know how to perform annotation, go to *Chapter 4, Annotations* .

The SHAPE ANALYSIS tab provides an easy to use interface to compute and record classical shape analysis methods (surface, Fourier parameters…). To know how to perform shape analysis, go to *Chapter 5, Shape analysis (Expert Mode)* .

The LIVE tab provides a way to perform annotation and acquisition at the same time. To know how to use the live mode, go to *Chapter 6, Live (Expert Mode)* .

The APPRENTICESHIP OF INDIVIDUAL AGE tab provides ways to learn how to automatically estimate the age of a species from calcified structures whose age have been estimated by the reader. To know how to use the apprenticeship of individual age, go to *Section 7.1, "Apprenticeship of individual age"* .

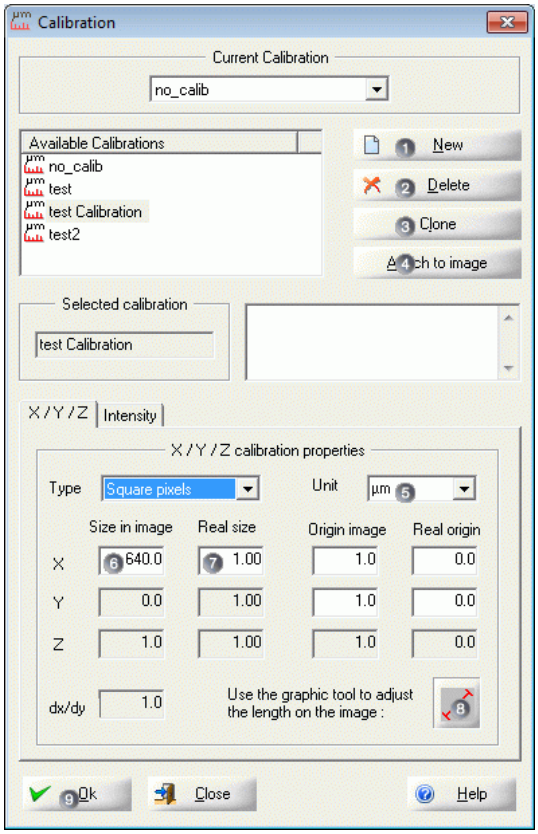
The AUTOMATIC ESTIMATION OF INDIVIDUAL AGE tab allows you to automatically estimate individual age of calcified structures. To know how to use the automatic estimation, go to *Section 7.2, "Automatic estimation of individual age"* .

The AUTOMATIC ESTIMATION OF AGE STRUCTURE tab provides features to automatically estimate the age structure of a database. To know how to use the automatic estimation of age structure, go to *Section 7.3, "Automatic estimation of age structure"* .

1.4. Perform a calibration

The best way to perform a calibration is to put a micrometer, or an object you know precisely the length, on your stage and take a picture of it. Then, click on to open the CALIBRATION window.

Figure 1.6. Calibration window

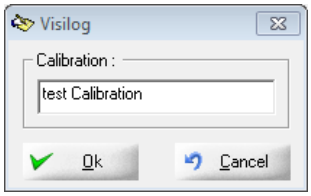


1. Create a new calibration.
2. Delete a calibration.
3. Create a new calibration from an existing one.
4. Attach the calibration to the current image.

5. The measure unit.
6. The length of the measure in pixels
7. The real size of the measure in the unit defined.
8. Draw the measure on the current image.
9. Validate.

To create a new calibration, click on New. You will be asked to enter a name for your calibration.

Figure 1.7. Enter new calibration name



Click on to display the measure bar on the picture. Move it so that the distance measured is the one of the micrometer, or the object. Then, in the CALIBRATION window, you should see the length in pixels. Put in front of it the real size and its unit.

Tip

For a better precision, your micrometer, or object, should be on the X axis.

Note

You should perform a calibration for each of your microscope lenses.

Note

This is a basic explanation about calibration. If you want more information about it, please refer to the Visilog 6™ User Guide.

1.5. Settings management

When you perform a lot of different otholith analysis, you might need to change settings, save them, load old settings…

Each time you get settings, you should have all those possibilities in the settings toolbar.

Figure 1.8. Settings toolbar

1. Create a new set of settings.
2. Load a set of settings.
3. Save the set of settings.
4. Save the set of settings under an other name.
5. The name of the set of settings used.

Note

If you work on a species and you got a set of settings named the same way (must be the exact same spelling, for example 'Albacore'), this set of settings will be loaded at startup. This might be useful when you always work on the same species and got a set of settings per species.

Note

You might want to save your settings in case you change computer… To do so, save the settings `.cfg` files on any support you want (USB key, CD, HDD…). The files are located :

- on Windows XP on : `C:\Documents and Settings\All Users\Documents\Noesis\Tnpc`
- on Windows Vista or Windows 7 on : `C:\Users\Public\Documents\Noesis\Tnpc`

Chapter 2. Databases

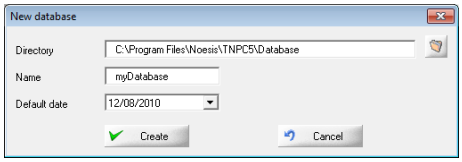
2.1. Introduction

TNPC facilitates the creation and the analysis of CS (otoliths, scales…) image databases. You can perform process on all the database including automatic exports of the data without any click or keyboard use. To have a full description of the database functions, please consult the Visilog 6™ Database Module documentation.

2.2. Create a database

To create a database you have to go to the DATABASE tab. Click on FILE → NEW DATABASE. You then have to select the directory in which the database will be stored, its name and the default date.

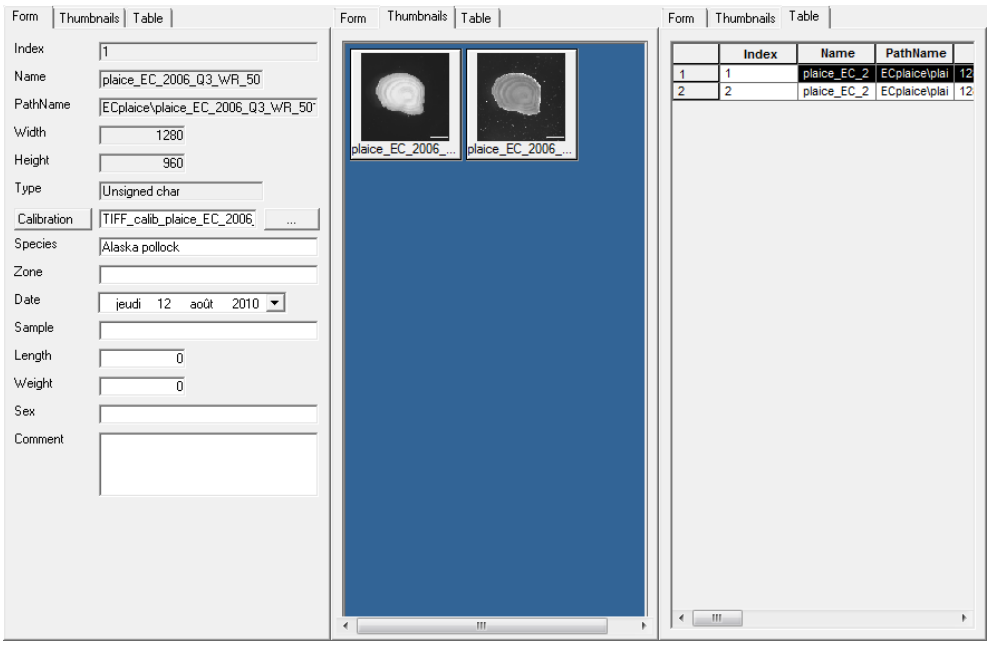
Figure 2.1. The New database Window



The database has by default 9 fields : Calibration, Species, Zone, Date, Sample, Length, Weight, Sex, Comment. You can add other custom fields (to do so refer to the Visilog 6™ Database Module documentation).

You can visualize your database three different way : forms (by default), list of thumbnails or table.

Figure 2.2. Database views



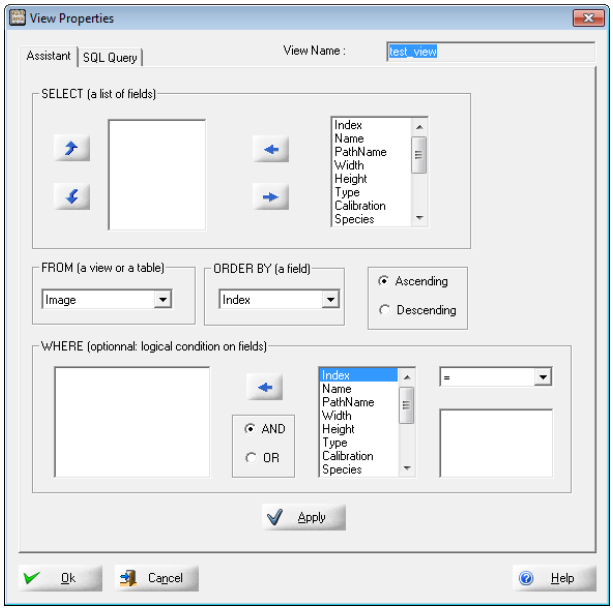
Note

The list of thumbnails visualisation might be unactivated. To activate it, go to OPTIONS → SETTINGS and click on Display All on the THUMBNAIL MANAGEMENT section.

2.3. Manage views

Queries or views in the database can be written using a form or plain SQL in the VIEW PROPERTIES window. For more information about how to write queries, please refer to the Visilog 6™ Database Module documentation.

Figure 2.3. The View Properties window



You can create a view from scratch by clicking on VIEWS → NEW DATABASE VIEW. You will be asked to enter a name for your view and then will be redirected to the VIEW PROPERTIES window.

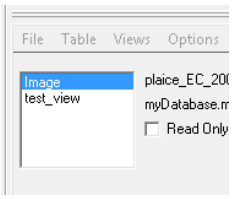
To simplify view creation, you can also create a view by cloning the current view using VIEWS → CLONE CURRENT VIEW. You will be asked to enter a name for your new view and then will be redirected to the View Properties window.

You can modify the current view by clicking on VIEWS → MODIFY CURRENT VIEW. You will be redirected to the View Properties window.

You can delete the current view by clicking on VIEWS → DELETE CURRENT VIEW. You will be asked for a confirmation.

We have just seen than you can create several views and that operations are always done on the current view. To change view, you have to select the view you want in the view list, on the top of the DATABASE tab.

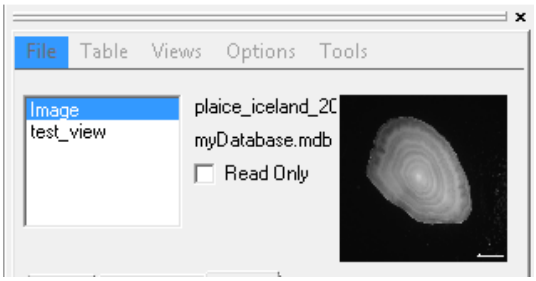
Figure 2.4. Database views list



2.4. Display information and images from a database

To display an image from a database, you have to select it and then double click on its thumbnail. It will load it as the current image. Interpretations and analysis can be loaded by clicking on the appropriate fields.

Figure 2.5. Image thumbnail

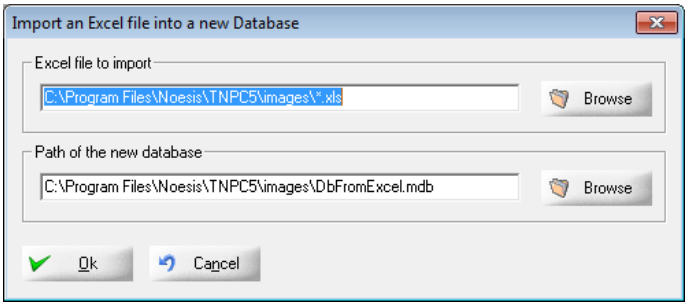


2.5. Import database from an xls file

You can create a database from an xls file (coming from a spreadsheet software such as OpenOffice Calc or Microsoft® Excel™).

To do so, click on FILE → IMPORT EXCEL FILE. You will have to choose the file you want to import and the database you want to create. If the columns exists in TNPC, they will be filled with the data present in the database. If columns do not exist in the database, they will be added.

Figure 2.6. Create a database from an xls file



Warning

This way, you cannot add data to an existing database, the import will create a new database.

2.6. Add images

You have three ways to add images to a database, you can add the current image, an image or a folder of images.

FILE → ADD CURRENT IMAGE

Will add the current image to the database (The button at the bottom of the database tab will do the same).

FILE → ADD IMAGE FILE

Will add an image file to the database. You will chose the file and click on Open.

FILE → ADD A FOLDER

Will add all the images of a folder to the database. You will chose the folder and click on OK

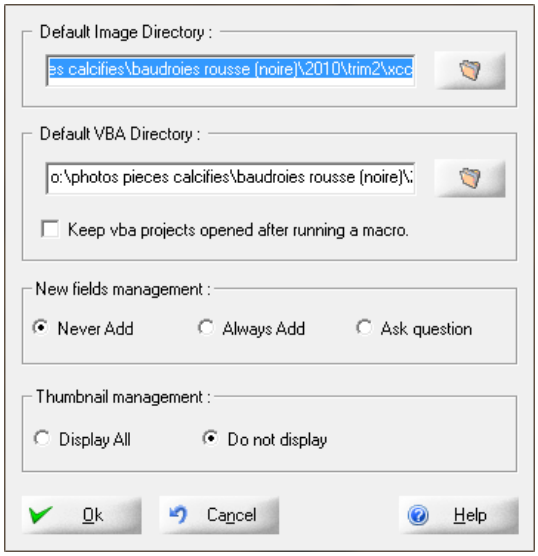
Note

You might be asked to add the new fields to the database. Those fields are the fields from the image (resolution…). You can go to the options to always answer yes or no.

2.7. Move a database with its images

To move a database with its images, you should open it in TNPC, and then change the DEFAULT IMAGE DIRECTORY and DEFAULT VBA DIRECTORY in the DATABASE SETTINGS window. You can open this window by clicking on OPTIONS → SETTINGS

Figure 2.7. The database settings window



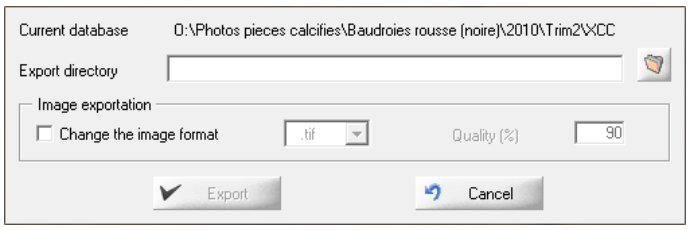
Note

It is also possible to modify the paths by opening the database with Microsoft® Access™ before opening it in TNPC.

2.8. Modify a database images format

It might be useful, to change the images format, so that people that do not have TNPC can read them. To do so, export the images using TOOLS → EXPORT. Choose here the new file format and select the parameters depending on the format.

Figure 2.8. The database exportation window



Chapter 3. Image acquisition

3.1. Features

You can acquire images using different ways. You can acquire an image from a microscope connected to your computer. If this microscope is equipped with a motorized stage, you will be able to make mosaics from this microscope. You can also acquire mosaics of slices and separate the otoliths. If you got a scanner, you can also acquire otoliths using it.

3.2. Configure stage, microscope and autofocus

Note

This part is useful only for Expert mode users. For routine mode users, you can go directly to the *settings part* .

Before using the acquisition for the first time, you have to initialize the stage controller (if any), the microscope and the autofocus by defining certain elements for each in the STAGE tab.

To access those settings, you have to open the PARAMETERS window by clicking on  . A window with four tabs appear. The first tab is about trajectory parameters, do not focus on it, first you have to configure you hardware.

3.2.1. Initializing the stage controller

Note

If you do not have motorized stage, you can skip this part.

Initializing the stage controller consist in establishing the communication between the controller and the physical parameters of the system. To access those parameters, select the STAGE tab.

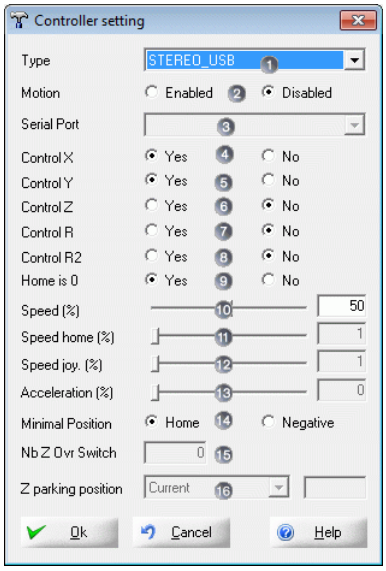
3.2.1.1. Controller settings

You first have to set the configuration of the electro-mechanical system (type of stage, COM port, etc.) in the CONTROLLER SETTING window. To access it, select the number of controllers, your controller type in the drop-down list and click on  .

Note

If you do not find your controller in the drop-down list, please contact Noesis company.

Figure 3.1. Controller settings



1. The type of your controller. If you do not find your controller in this list, please contact Noesis.
2. Whether the controller is initialized or not. You must choose disabled if you do not have a controller (as in Demo mode) or if it is not connected.
3. The port used.
4. If the X axis is controlled.
5. If the Y axis is controlled
6. If the Z axis is controlled.
7. If the R axis is controlled.
8. If the R2 axis is controlled (second rotation).
9. Whether the Home position is the same as the reference position.
10. The movement speed.
11. The speed to go to the Home position.
12. The speed when using the joystick.
13. The stage acceleration.
14. Whether the minimal position is the Home position or the controller accepts negative values.
15. The number of electronic stops along the Z axis.
16. If you want the Z axis to stop at a certain point when TNPC close. Use this if you do not have electronic stops along the Z axis. You can still use it if you got electronic stops.

Note

You can find all those information in your controller and camera/microscope manual. If those information are not present, please contact your manufacturer to get them.

3.2.1.2. Stage parameters

When your controller is set, you can define the stage parameters. You got four tabs for the minimum position, maximum position, center position and motor parameters.

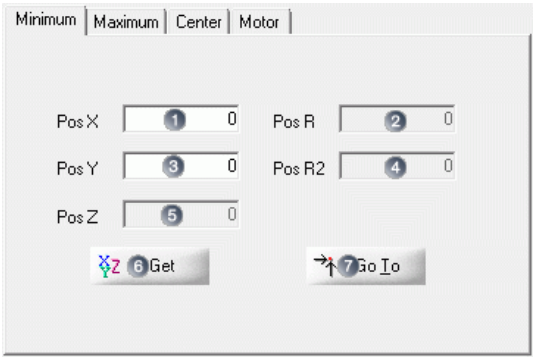
3.2.1.2.1. Minimum position

The minimum position defines the software-based minimum stop positions (in motor step sizes) for the X, Y, Z and R axes. Select an axis and enter a value in the field. To be more precise, or if you do not know what value to enter, you can select the current position by clicking Get. To automatically search for the minimum position, click on Go To.

Warning

Do not use Go To if one of your axis has no mechanical stops, that could damage your motorized stage and your microscope.

Figure 3.2. Minimum position tab

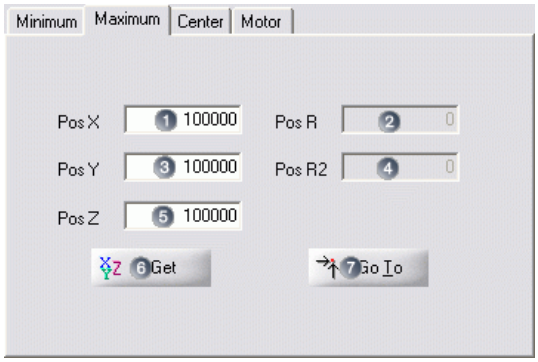


1. The X coordinate of the minimum position.
2. The R coordinate of the minimum position.
3. The Y coordinate of the minimum position.
4. The R2 coordinate (second rotation) of the minimum position.
5. The Z coordinate of the minimum position.
6. Button to acquire the actual position coordinates.
7. Button to automatically go to the minimum position on each axis.

3.2.1.2.2. Maximum position

The maximum position defines the software-based maximum stop positions (in motor step sizes) for the X, Y, Z and R axes. Select an axis and enter a value in the field. To be more precise, or if you do not know what value to enter, you can select the current position by clicking Get.

Figure 3.3. Maximum position tab

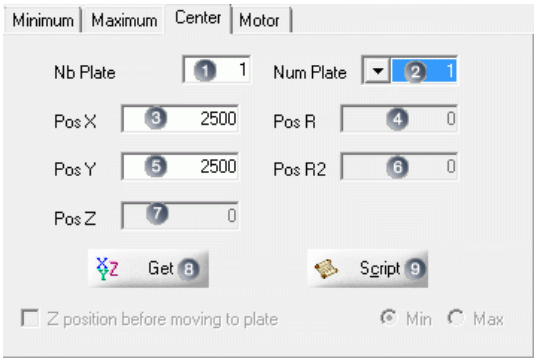


1. The X coordinate of the maximum position.
2. The R coordinate of the maximum position.
3. The Y coordinate of the maximum position.
4. The R2 coordinate (second rotation) of the maximum position.
5. The Z coordinate of the maximum position.
6. Button to acquire the actual position coordinates.
7. Button to automatically go to the maximum position on each axis.

3.2.1.2.3. Center position

Center is an optional parameter that lets you establish the center of the X, Y, Z and R axes. You can define as many centers as you like. For instance, if the stage contains multiple slides, you can define a center for each. Make sure that you have correctly selected the center index before entering its position. For a single slide, you can move the stage to a particular position and click Get. Either way, use Script to define a center serial.

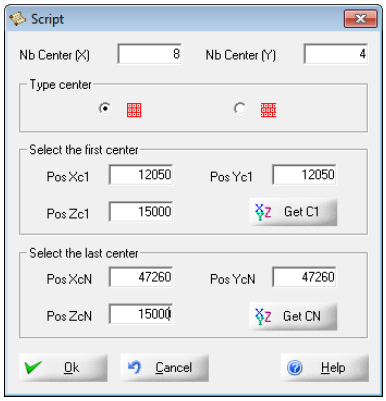
Figure 3.4. Center positions tab



1. The number of plates on the stage.
2. The number of the plate you are editing.
3. The X coordinate of the plate center position.
4. The R coordinate of the plate center position.
5. The Y coordinate of the plate center position.
6. The R2 coordinate (second rotation) of the plate center position.
7. The Z coordinate of the plate center position.
8. Button to acquire the actual position coordinates.
9. Button to define a center serial.

If you want to use a script to define a center serial, please refer to the Visilog 6™ Stage module documentation, page 1-12.

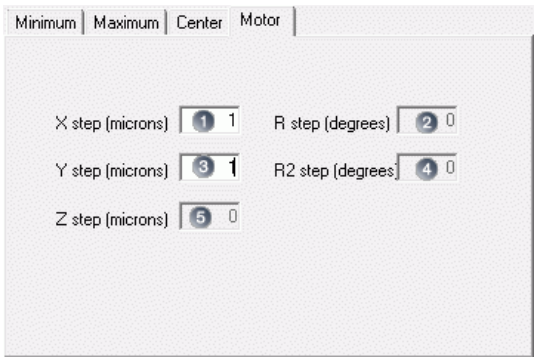
Figure 3.5. Center serial script window



3.2.1.2.4. Motor parameters

In the motor parameters tab, you have to enter the step size for each motor. Once these values are set, the jog menu will automatically have distance steps available such as 1µm, 10µm, etc. By default, the stage controller assumes that all measurements are in microns. Once you have set all other measurements and calibrations (for autofocus, etc.), the measurement units are automatically updated.

Figure 3.6. Motor tab



1. The length (in microns) the plate moves on the X axis for each motor step.
2. The angle (in degrees) the plate rotates on the R axis for each motor step.
3. The length (in microns) the plate moves on the Y axis for each motor step.
4. The angle (in degrees) the plate rotates on the R2 axis (second rotation) for each motor step.
5. The length (in microns) the plate moves on the Z axis for each motor step.

3.2.1.2.5. Saving parameters

The previous steps are mandatory to initialize the stage controller. Once you have completed them, use Save to save the configuration on the disk in the file `plugins/services/stage/stage.cnf`. Use Reset to load a previously saved configuration.

3.2.1.3. Microscope parameters

Initializing the microscope consist in establishing its physical characteristics. You can define settings for each lens, such as the size of each field in motor steps, or the range in motor steps for the autofocus. Once those parameters have been defined, moving the stage from one field to the next takes only a mouse click. To open the microscope settings tab, click on the MICROSCOPE tab.

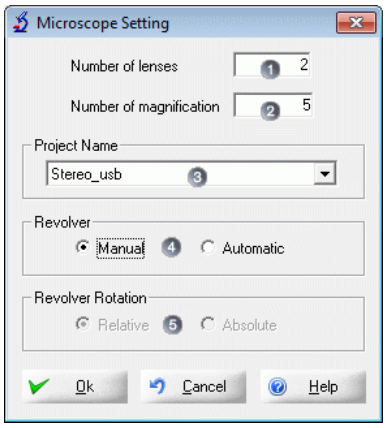
Note

The stage module can remotely control revolving lenses, and so change the lenses automatically if your microscope support this feature. However, you should have a look to the Visilog 6™ Stage Module documentation page 2-23 for more details.

3.2.1.3.1. Microscope settings

To open the microscope settings window, click on Setting button.

Figure 3.7. Microscope settings



1. Number of different lenses on your microscope.
2. The number of magnification on your microscope.
3. Choose here the project corresponding to your controller. If your controller is not present, please contact Noesis.
4. Whether the lens change is manual or automatic.
5. When the lens change is automatic, whether the revolver rotation is relative or absolute.

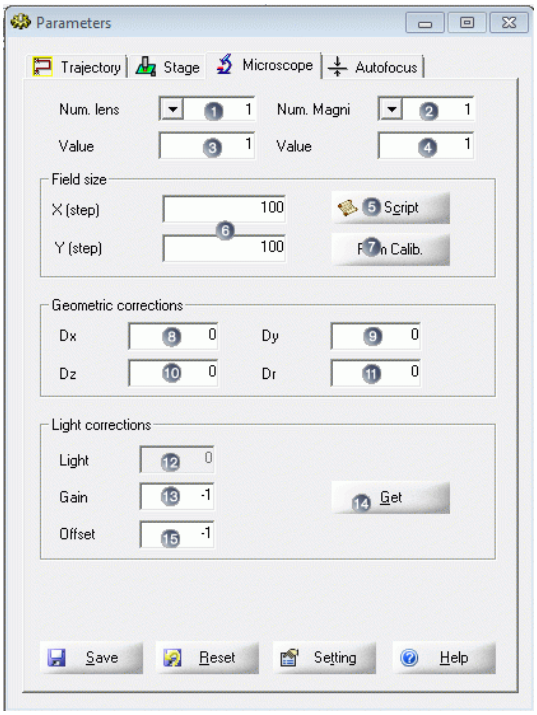
> **Note**
>
> If you do not have a microscope, you can use these fields to define the fields of view and autofocus range. In which case, define only one lens and a single magnification.

Once everything is set, click Ok and return to the MICROSCOPE PARAMETERS tab.

3.2.1.3.2. Microscope parameters

In this tab, you can define, for each lens/magnification combination, the lens, field size, autofocus steps and camera parameters. Make sure that you have correctly selected the lens and magnification before entering its parameters.

Figure 3.8. Microscope parameters



1. The number of the lens you are editing.
2. The number of the magnification you are editing.
3. The value of your lens.
4. The value of your magnifier.
5. Use a script to determine the field size.
6. The field size in motor steps.
7. Use a calibration to determine the field size. To know how to perform a calibration, go to *Section 1.4, " Perform a calibration"*
8. Geometric correction on the X axis.
9. Geometric correction on the Y axis.
10. Geometric correction on the Z axis.
11. Geometric correction on the rotation axis.
12. Light correction value. If -1 is selected, the default value will be used.
13. Gain. If -1 is selected, the default value will be used.
14. Get the light correction parameters from the current values.
15. Offset. If -1 is selected, the default value will be used.

3.2.1.4. Autofocus parameters

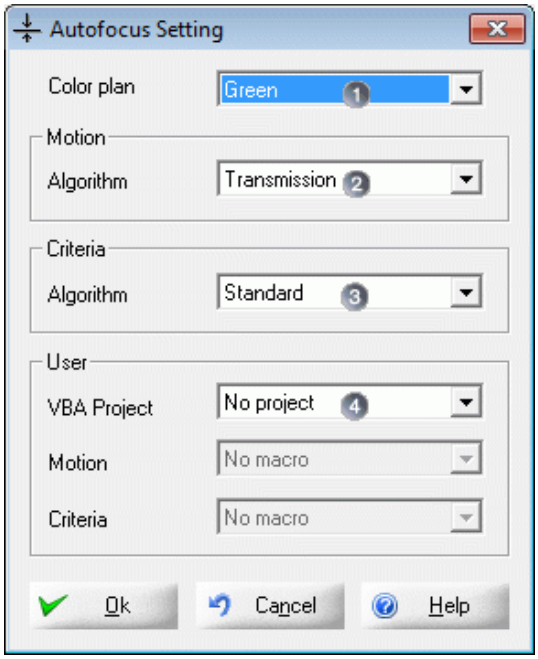
Autofocus provides a clear and focused image by moving the microscope stage along the Z axis. This presumes that the controller has a Z-axis control and that it has been initialized. Stable parameters, such as the autofocus algorithms, are in the AUTOFOCUS SETTING window. While parameters subject to

frequent modification, such as those depending on the current objective or the current sample, are in the AUTOFOCUS tab. If you want to understand better how autofocus works, please refer to the Visilog 6™ Stage Module documentation.

3.2.1.4.1. Autofocus settings

To open the autofocus settings window, click on Setting.

Figure 3.9. Autofocus settings

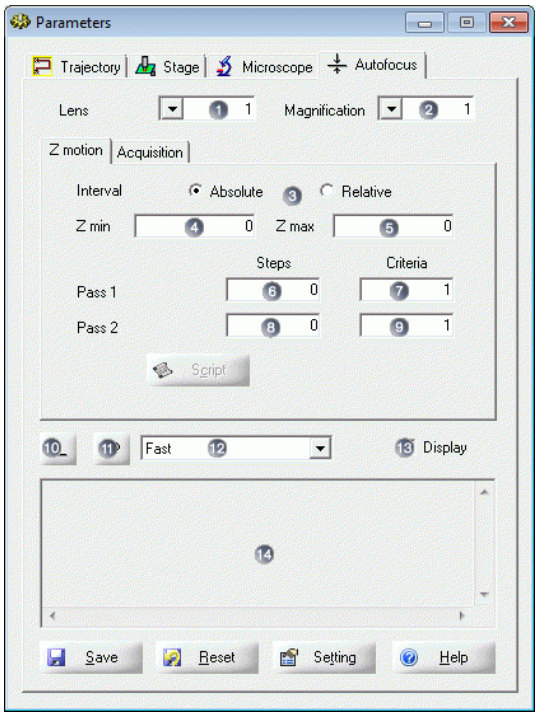


1. Choose here the color plan on which to perform the autofocus. If you have a color camera, you will save time if the autofocus is performed on a single channel (red, green or blue) or on a reduced part of the image. This is particularly important if you are working in reflection mode, because, unlike of transmission mode, you will always have an image no matter what is on the stage.
2. The motion default autofocus parameter corresponding to your hardware light source : transmission or reflection.
3. The criteria algorithm used on each pass. The default one is Standard. If you want to change this parameter, please refer to the Visilog 6™ Stage Module documentation.
4. Options to define your own autofocus algorithm. If you want to define your own autofocus algorithm, please refer to the Visilog 6™ Stage Module documentation.

3.2.1.4.2. Autofocus parameters

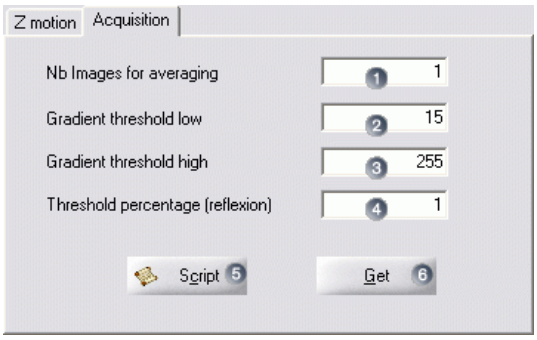
This tab allows you to establish the criteria for autofocus calibration. The parameters you select are defined each of the lens/magnification combination.

Figure 3.10. Autofocus parameters - Z motion tab



1. The number of the lens you are editing.
2. The number of the magnifier you are editing.
3. Whether the upper and lower limits of the autofocus range are defined in absolute or relative coordinates. If relative coordinates is selected, a mean Z value is used in the pad center.
4. The lower limit when in absolute coordinates or the mean value when in relative coordinates. Value is in motor steps.
5. The upper limit when in absolute coordinates or the delta value when in relative coordinates. Value is in motor steps.
6. The number of motor steps between scans. If the value is too large, you risk missing the ideal point.
7. The minimal score for each scan. It must correspond to the number of pixels considered as clear cut by the criteria calculation algorithm.
8. When to stop searching for the best possible image by establishing a threshold of motor steps between the two best images. If the steps are two small, the autofocus takes longer than necessary, if they are too large, the final point will not be accurate. In practice, it should be less than the depth of field.
9. The minimal score for each scan. It must correspond to the number of pixels considered as clear cut by the criteria calculation algorithm.
10. Test the selected parameters.
11. Clear the output text (see 14).
12. Select the output mode, Fast will just display the results, Verbose will display a lot of information like the value used in the algorithms. The autofocus calculation might take longer to proceed.
13. If checked, the intermediary images are displayed in the viewer area to help you understand the operation of the algorithm.
14. The output text.

Figure 3.11. Autofocus parameters - Acquisition tab



1. The number of images used for averaging in each scan.
2. Low gradient threshold (Used by the criteria calculation algorithm).
3. High gradient threshold (Used by the criteria calculation algorithm).
4. The percentage of the maximum criteria from which the first pass will stop when using a reflected source.
5. Use a script to adjust parameters 2 and 3
6. Get the actual values.

3.2.2. Try the settings - First Use

This step might seem a bit tedious, but it is necessary to ensure your microscope and motorised stage are well configured.

3.2.2.1. Standard acquisition

The first thing to check is that you can perform a standard acquisition. This should always be fine, but you need to check it first before trying the stage. To know how to make a standard acquisition, refer to *Section 3.4, "Standard acquisition"* . Check that the image you acquired is the one you wanted. If so, you can try making your first mosaic image. If not, please configure again your microscope.

3.2.2.2. Mosaic acquisition

You will then check that your motorised stage is well configured by performing a mosaic image. To know how to make a mosaic using a trajectory, refer to *Section 3.5.1, "Make a mosaic from a trajectory"* . Check that the image you acquired is the one you wanted. If not, please configure again your microscope and stage, it this happens again, refer to the troubleshooting section.

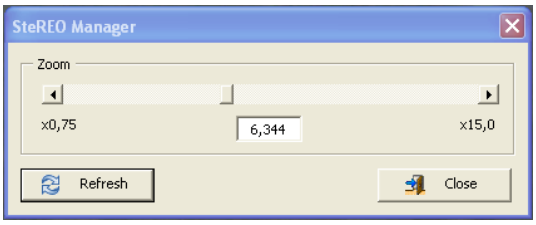
3.2.2.3. Troubleshooting

When your mosaic does not look like what you are expecting, there are some basic operations you can do to try to fix things.

- If you have a specific software for your controller (for example if you have a Zeiss controller). You can try to switch axis (one or several).

- You can try to rotate the camera of 180°.
- Check that the camera is perpendicular to the motorized stage.
- Check that the stage is not tilt. You may want to use a spirit level.
- Take care of the zoom conversion between the command and TNPC. For example, with a Zeiss stereomicroscope, the zoom goes from 4.7x to 95x but in TNPC, it goes from 0,75x to 15x. Either calculate the right value for the zoom, or select the right zoom with the command and hit Refresh, TNPC will make the conversion for you.

Figure 3.12. Automatic zoom conversion



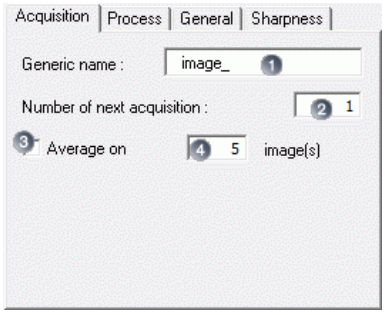
3.3. Settings

When you are on the TNPC screen, on the ACQUISITION tab, you can display/hide the settings by clicking on . There are four tabs for each settings section.

3.3.1. Acquisition

In this tab, you can parametrize the acquisition by stating the generic name (the base name of all the images acquired) and the number of the next acquisition (number that will follow the generic name). To avoid noise and increase sharpness, you can ask TNPC to make different images on the same point and store only one image that is an average of all the different images.

Figure 3.13. Acquisition parameters - Acquisition tab



1. The generic name used for acquisitions. All image names will start by this name and will be followed by a number.
2. The number of the next image acquired. In this example, the next image will be named image_1

3. Use an average on several images
4. The number of images on which to make an average if selected.

3.3.2. Process

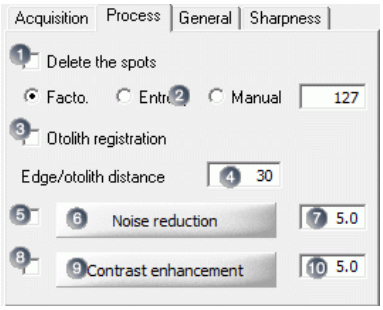
In this tab, you can specify a number of different processes to perform on the image to acquire in order to improve the image quality.

Note

The process is performed on each image acquired when acquiring slides.

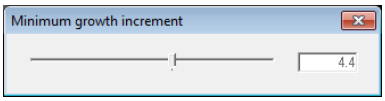
You can delete the spots, reduce image size to get only the otolith, configure the noise reduction and contrast enhancement algorithms.

Figure 3.14. Acquisition parameters - Process tab



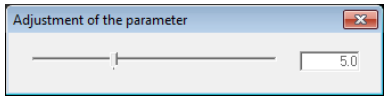
1. Function that detects the calcified structure in the picture and to remove the background (noise, artefact…). It relies on the image binarisation from a grey level threshold. This function will be efficient only is the observed object is contrasted compared to the background.
2. The spot deletion threshold configuration. It can be automatic using a factorial or entropic analysis of the grey-level histogram or manual (and you need to state a value).
3. This function rotates the image so that the otolith main axis is horizontal. It also crops the background to let a constant distance between the image and the otolith. This function needs to have the 'Delete the spots' function ran before.
4. The constant distance between the image and the otolith used in the otolith registration function.
5. This function reduce the noise in the image using a Gaussian filter parametrized by its variance.
6. Open the noise reduction parameter selection window. This parameter value is expressed in function of the smallest growth rings width. Using the bar, you can move the parameter value. The modification result is seen in real time on the window over the current image.

Figure 3.15. The noise reduction parameter adjustment.



7. You can also manually state the noise reduction parameter.
8. This function enhance the contrast (mostly on the edges). It relies on a Laplacian filter using a parameter.
9. Open the contrast enhancement parameter selection window. Using the bar, you can move the parameter value. The modification result is seen in real time on the window over the current image.

Figure 3.16. The contrast enhancement parameter adjustment.

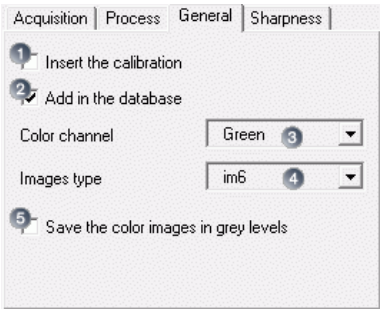


10. You can also manually state the contrast enhancement parameter.

3.3.3. General

In this tab, you can parametrize how the image is stored : its format, its color channel, if the calibration have to be inserted, if the image have to be added to the database, or if the image need to be stored in grey levels.

Figure 3.17. Acquisition parameters - General tab



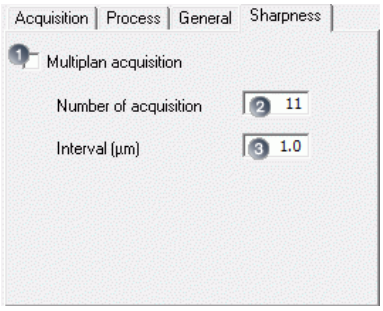
1. Insert the calibration in each image.
2. Add the acquired image in the database.
3. The color channel to use when you got a color camera.
4. The image format. For more info, read *Image format* .
5. Whether to save the color images in grey levels.

3.3.4. Sharpness (Expert Mode)

Warning

This tab is useful only if you got a stage with a motorized Z-axis.

Figure 3.18. Acquisition parameters - Sharpness tab



1. Make a multi-plane acquisition and calculate the best image.
2. The number of acquisition to perform around the current position for each image.
3. The interval length, on the Z axis, between each acquisition.

3.4. Standard acquisition

The standard acquisition process consist in acquiring what the camera can see. To acquire an image in standard mode, click on . The camera is now active and you can see what will be acquired in the main window. A new icon is shown in the tab : . Click on it to freeze the image you want to acquire. A window pops up. If you choose yes, all the processes you defines are then applied (see *Section 3.3.2, "Process"*). The resulting image is displayed in a pop-up window while the main window still shows what the camera sees for the next acquisition.

3.5. Mosaics (Expert mode)

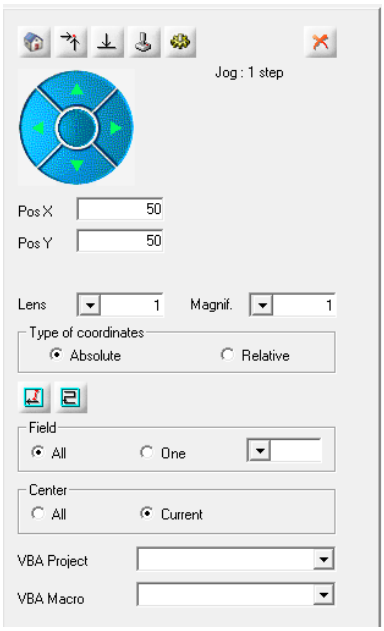
If the camera field does not cover the entire field of analysis, image mosaics can be acquired automatically using the controlled stage.

3.5.1. Make a mosaic from a trajectory

3.5.1.1. Build a trajectory

To build a mosaic, you first need to set a trajectory. To do so, go to the STAGE window.

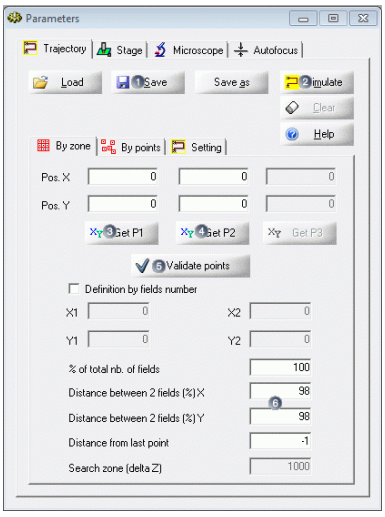
Figure 3.19. Stage window



At the beginning of the session, initialize the motorized stage by clicking on . The stage will move until it finds its origin value. Activate the joystick by clicking on . Click on to open the configuration window of the stage.

The trajectory will be calculated from a rectangle. You have to give the two opposite points of the rectangle so that the trajectory can be computed. Move the stage to the starting point (P1) of the mosaic with the joystick, then read P1 clicking on Get P1. Then move the stage to the ending point (P2) and click on Get P2. The distance between 2 fields must be 98% on the X and Y axis. In the SETTING tab, the field EDGE OVERLAPPING must be ticked. Visualize the trajectory by clicking on Simulate. The number of images and the digitized surface will be displayed. Save the trajectory and name it. The file will be saved in `Tnpc/Data/Trajectory` and will have a `.trj` extension.

Figure 3.20. Trajectory configuration

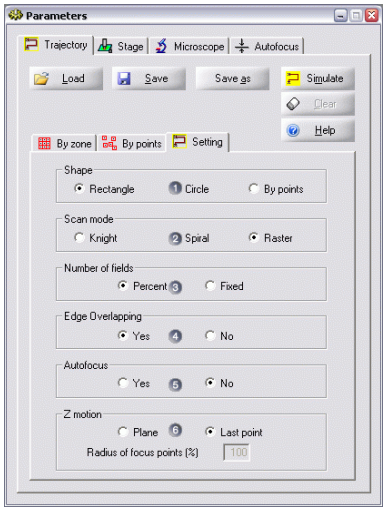


1. Save the trajectory.
2. Simulate the trajectory.
3. Get Point 1 coordinates. Above, you got the coordinates.
4. Get Point 2 coordinates. Above you got the coordinates.
5. Validate the acquired points.
6. Overlapping values. The default values should be fine, if you want to change those values, please refer to the Visilog 6™ Stage Module documentation.

Warning

The settings must always be configured as stated below. If you want to change those parameters, please refer to theVisilog 6™ Stage Module Documentation.

Figure 3.21. Trajectory settings

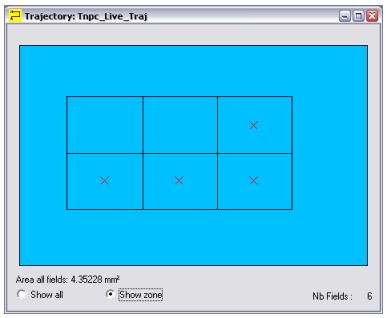


1. The shape should always be a RECTANGLE.
2. The scan mode should always be RASTER.
3. You should always put PERCENT here, or you can have holes in your mosaic.
4. Edges should always be overlapping.
5. Indicate if the focus is automatic or manual.
6. Do not change this parameter.

3.5.1.2. Make the mosaic

When you are back on the TNPC tab, you can launch the mosaic acquisition by clicking on . You will see a blue table whose cells correspond to an image. The stage will move and the images will be acquired one by one. You can follow the process on the table, a red cross appear on the cells that have been acquired.

Figure 3.22. Mosaic acquisition in progress



Warning

In order to get the mosaic acquisition to perform correctly, you need to have two images names RefBlanc and RefNoir loaded in TNPC. The RefBlanc and RefNoir images are used to fix the lighting drifts. If the acquisition system got no lightning defaults, then the RefBlanc picture can be full white and the RefNoir image can be full black.

3.5.2. Make a mosaic of a slice

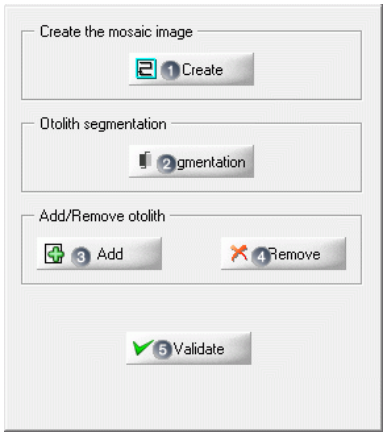
You might also want to make a mosaic of a slice and then get an image per otolith on the slice. There is two way to proceed. Either way, you first need to have a trajectory created (see *Section 3.5.1.1, "Build a trajectory"*).

The first way to proceed is automatic, TNPC acquires the mosaic and then perform the otolith detection. To launch the acquisition click on .

The second way to proceed is semi-automatic. Click on to open the menu. Click on Create to launch the mosaic acquisition according to the trajectory you created. Then, when the mosaic have been acquired, click on Segmentation to perform the otolith separation.

When all the otolith are segmented, click on Validate to perform all the processes that are configured (see *Section 3.3.2, "Process"*).

Figure 3.23. Semi-automatic slice acquisition

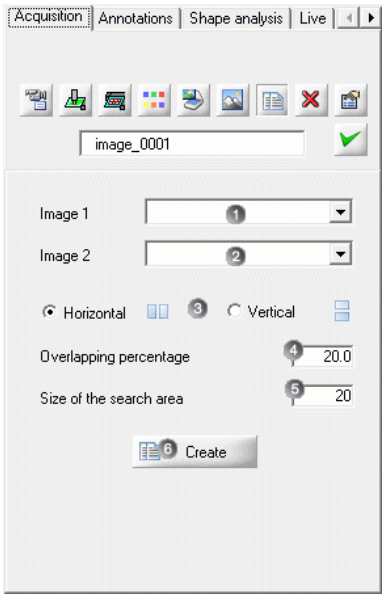


1. Create the mosaic image.
2. Launch the otolith detection.
3. Add an otolith to the automatic detection.
4. Remove an otolith from the automatic detection.
5. Validate the detected otolith and perform the processes.

3.5.3. Make a mosaic from two images

If you do not have a motorized stage, you still can make mosaics using two images. Open the interface clicking on . You then have to choose the two images (they need to already have been loaded in TNPC), state how to perform the mosaic and then click on Create.

Figure 3.24. Mosaic manual creation



1. The first image.
2. The second image.
3. Make the mosaic horizontal or vertical.
4. The overlapping rate between the two images.
5. The tolerance in the direction normal to the displacement (ie, horizontally when you select a vertical mosaic).
6. Launch the mosaic creation.

3.6. Image batches acquisition using scanner

When you do not have camera and stage, you can use scanners to acquire images. Scanner can allow you to acquire either images batches that will then be separated or images alone.

Warning

Take care about this acquisition mode as the precision might not be enough to make interpretations on the image after acquisition.

To perform a scanner acquisition, click on  . A window will pop-up allowing you to choose between the scanners installed on your computer. Choose the scanner you want to use and click on Select.

Note

To be used in TNPC, your scanner have to be installed on the computer first. To do so, please refer to your scanner manual.

Note

On a scanner acquisition, you can acquire several slices at the same time. Just bear in mind that the quality might be poorer than using motorized stage and microscope. TNPC will split the otolith from the slices to get one otolith per image. To get a good split, dispose your slices like on a grid, with some space between each slice.

Use your scanner software to select the area to acquire and launch the scan. Once the scan is performed, TNPC get it back.

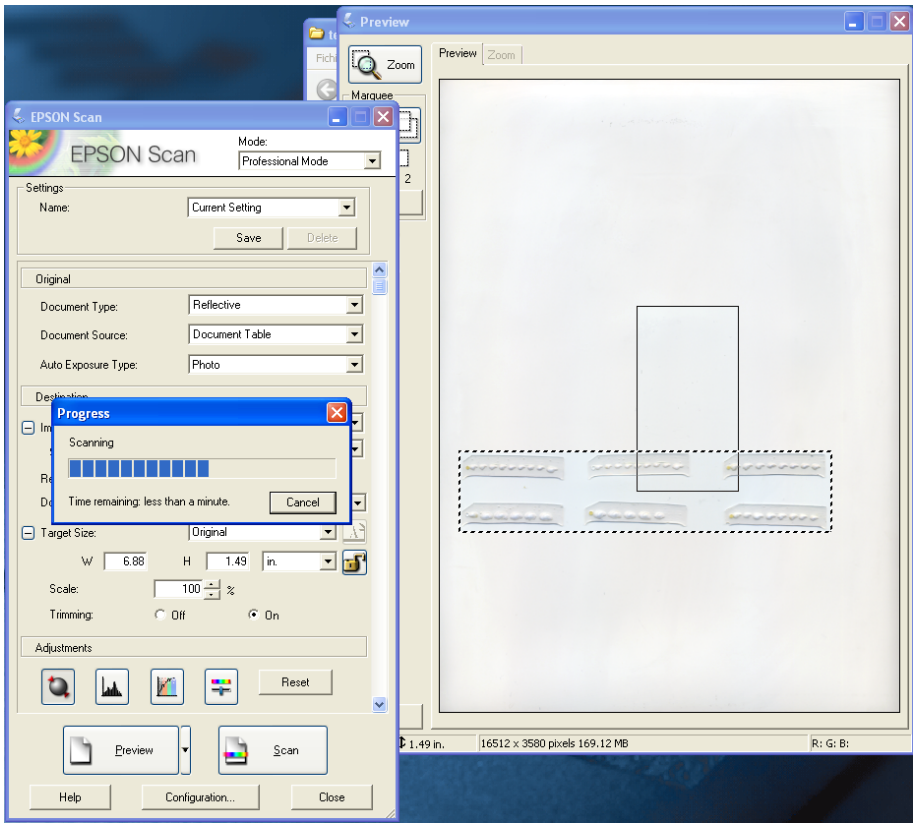
Warning

Acquisition might be limited by your computer RAM.

Note

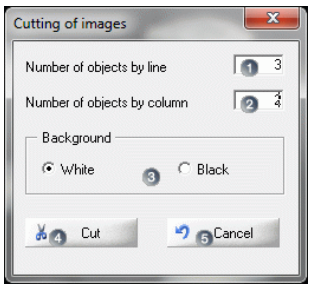
The otolith detection is always performed from the top of the image. If all the otoliths are at the same height, the detection is made form right to left.

Figure 3.25. Acquisition in scanner software



You have to tell TNPC how many slices there are on each row and each column of your grid.

Figure 3.26. Parametrizing otolith separation during scanner acquisition



1. Number of slices per row.
2. Number of slices per column.
3. The color of the background between slices, white or black.
4. Launch the cut (TNPC will try to cut all the different otoliths to got one otolith per image).
5. Cancel the cut, the acquired image will not be stored but will be kept as current image.

TNPC now split the acquired image into one image per otolith.

3.7. Current image processing (Expert mode)

When you have images that have already been acquired but of poor quality, you might want to use the acquisition processing tools to improve their quality. To do so, open the image you want to improve, select the process parameters you want to apply on the acquisition settings (cf. *Section 3.3.2, "Process"*).

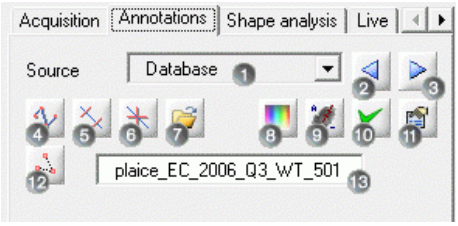
You can then click on  and all the process you have set will be applied.

Chapter 4. Annotations

4.1. Generalities

The ANNOTATIONS section allows the user to interpret CS by creating radials, positioning markers or calculate markers positions using grey levels curves. The values can be saved to exchange with other people or for later use.

Figure 4.1. Annotations tab



1. The image source
2. Previous image (only when using database source)
3. Next image (only when using database source)
4. Create radial
5. Add a GR marker (Growth)
6. Add a CH marker (Check)
7. Open a radial. If the image name is filled-in (cf. 13), the radial will directly open. If not, you will have to select the radial file manually.
8. Choose the radial colors
9. Fusion image and annotations
10. Validate analysis for this image, go to next image when using database source
11. Settings
12. Create a radial with a rotation angle (cf. *Section 4.2, "Settings"*).
13. Name used for data export. By default, this is the image name.

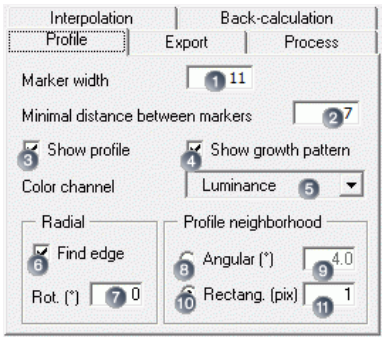
4.2. Settings

You can display/hide the settings by clicking on . There are five tabs for each settings section.

4.2.1. Profile

This tab allows you to parametrize the profile used for all the age calculation and processes. You can choose to visualize or not the intensity profile and the growth pattern, modify the markers size, the color channel used, the neighbourhood to calculate the intensity profile reducing noise…

Figure 4.2. Profile settings

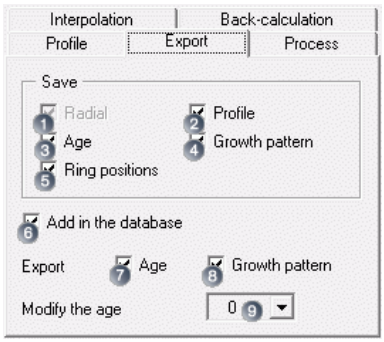


1. The width of the markers.
2. The minimal distance between markers.
3. Activate the intensity profile visualisation.
4. Activate the growth pattern visualisation.
5. Choose the color channel used : red, green, blue or a balanced mean of the three colors : the luminance.
6. Activate the edge detection when drawing radials (by default, the edge is detected).
7. Angle of rotation of the radial when pressing the button.
8. Activate the angular profile neighborhood.
9. The angle of the angular sector of the neighborhood.
10. Activate the rectangular box neighborhood.
11. The width of the rectangular box of the neighborhood in pixels.

4.2.2. Export

In this tab you can manage all the export settings.

Figure 4.3. Export settings



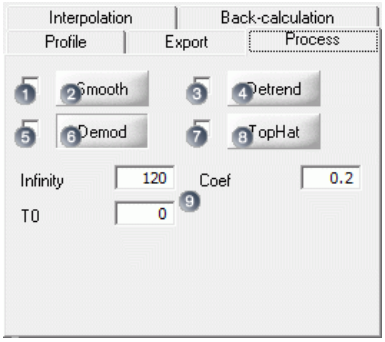
1. Store the radial in a `.rad` file located in the `rad` folder.
2. Store the intensity profile in a `.pro` file located in the `pro` folder.
3. Store the estimated age.
4. Store the growth pattern in a `.iid` located in the `iid` folder.

5. Store the rings positions in a `.xyd` located in the `xyd` folder.
6. Store the interpretation in the database.
7. Store the estimated age in a datasheet in the graphic window.
8. Store the growth pattern in a datasheet in the graphic window.
9. Add/remove one to the number of rings for age calculation or do nothing.

4.2.3. Process (Expert Mode)

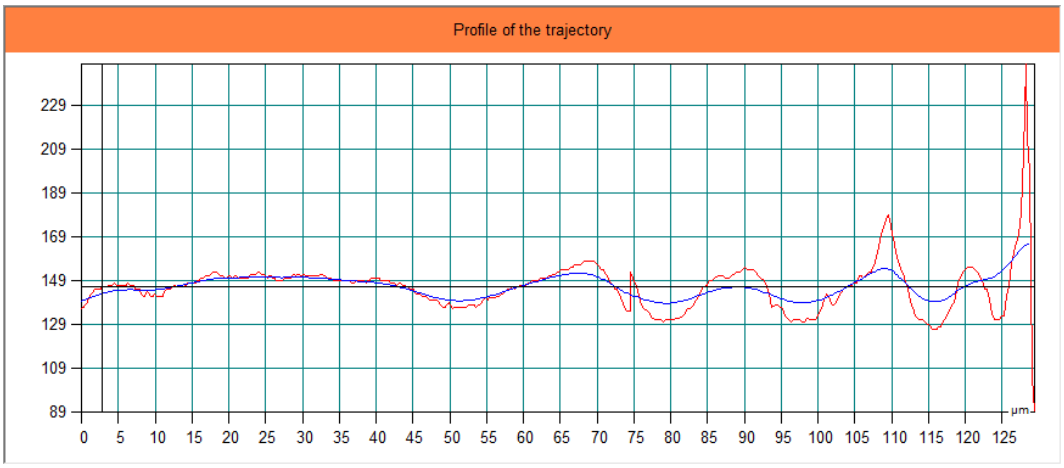
On this tab, you can manage all process settings : which processes will be performed and what are their parameters.

Figure 4.4. Process settings



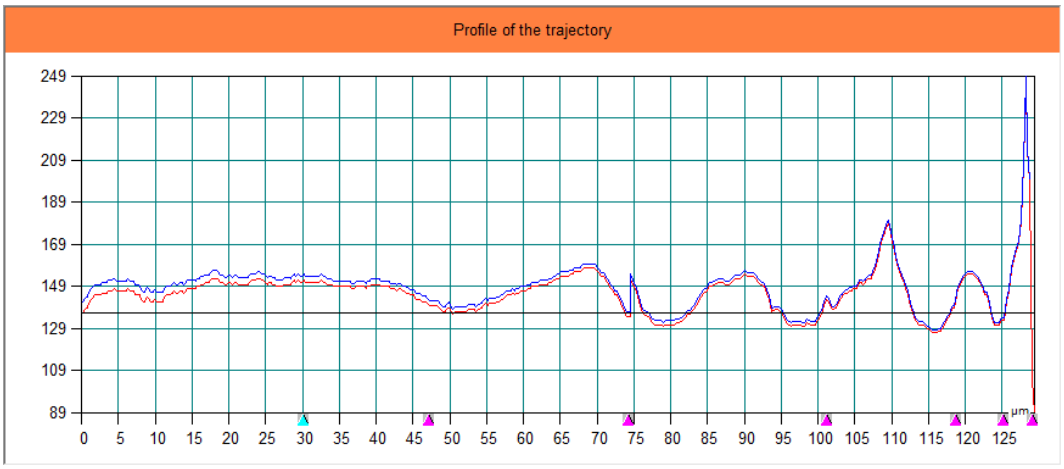
1. Activate the SMOOTH function. This function smooth the intensity profile to remove the high frequencies (noise, details…) from it. This function got only one parameter : the value of the thinnest ring width (the default value is 5). The value is in pixels if there is no calibration, in the calibration unit if it has been defined.

   Figure 4.5. Use 'Smooth' function - Before (red) - After (blue)



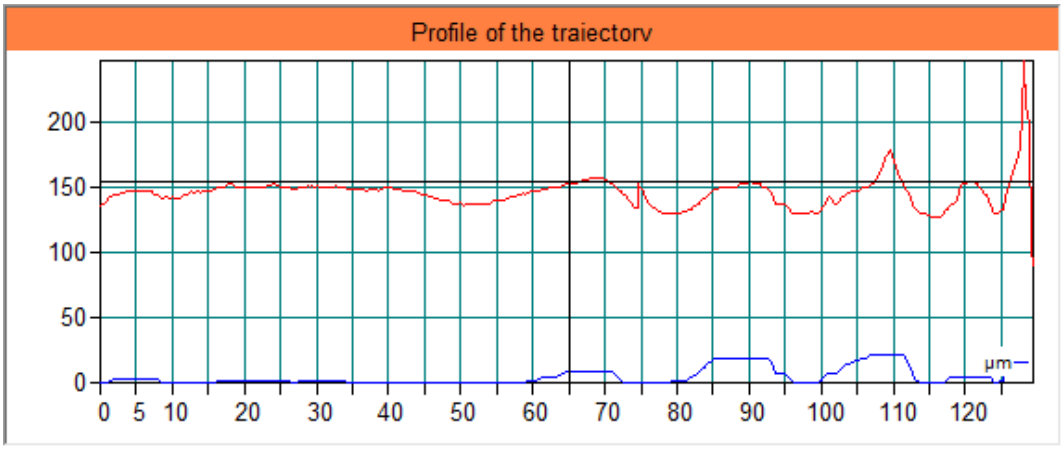
2. Show/Hide the SMOOTH function parameter.
3. Activate the DETREND function. This function removes the intensity profile trend. It is defined by one parameter : the largest growth ring width value (by default 50). The value is in pixels if there is no calibration, in the calibration unit if it has been defined.

Figure 4.6. Use 'Detrend' function - Before (red) - After (blue)



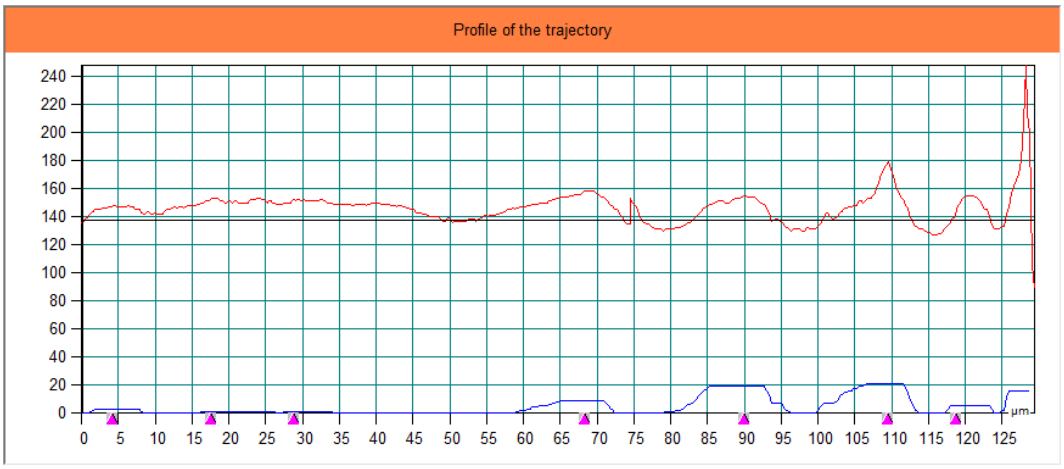
4. Show/Hide the DETREND function parameter.
5. Activate the demodulation function. This function creates a demodulation of the intensity profile using an a priori growth model. The intensity profile is seen as the result of the modulation of a periodic signal (daily or yearly) modulated by the fish's growth pattern. To be used, the DEMOD function needs to have the growth pattern values defined such as L_∞ (which default value is 120) and K (which default value is 0.2) for the Von Bertalanffy model, and L_0 and K for the exponential model. The first model is available only if the back-calculation function is activated (see *Section 4.2.5, "Back-calculation"*), the exponential model is always available.

Figure 4.7. Use 'Demod' function - Before (red) - After (blue)



6. Show/Hide the DEMOD function parameter.
7. Activate the TOPHAT function. This function detects the relevant peaks (by default) or valleys of the intensity profile. You need to set the minimum (default 5) and maximum (default 50) width of the structures to be detected and the threshold (default value is 0) for the 'hat' height. The markers are positioned on the peaks or valleys' extremum (by default), or on the end, beginning or barycenter of those peaks or valleys

Figure 4.8. Use 'TopHat' function - Before (red) - After (blue)

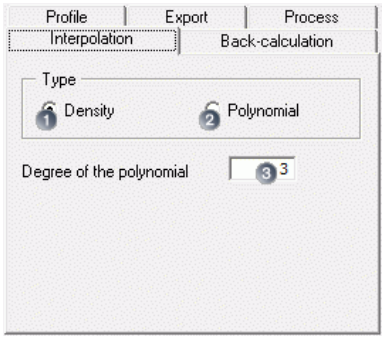


8. Show/Hide the TOPHAT function parameter.
9. The functions parameters.

4.2.4. Interpolation (Expert Mode)

This tab let you parametrize the interpolation function. This function is used to interpolate the number of growth increments within unreadable areas. You can choose between the density interpolation, that relies on a mean-frequency computed within the specified surrounding regions, or the polynomial interpolation, an implicit polynomial function, whose zero crossings are the positions of the growth markers within the specified surrounding region.

Figure 4.9. Interpolation settings

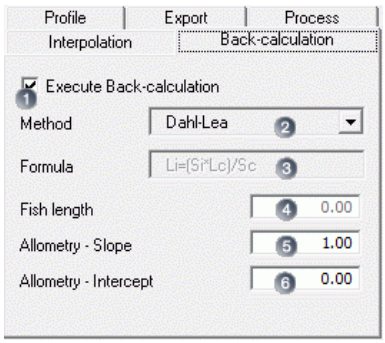


1. Activate the density interpolation.
2. Activate the polynomial interpolation.
3. The degree of the polynomial interpolation.

4.2.5. Back-calculation

This tab will allow you to activate or not and parametrize the fish's length back-calculation. To use the back-calculation function, the field "Length" must exist in the database. If it does not exist, you have to create it or rename the according field.

Figure 4.10. Back-calculation settings



1. Activate the back-calculation.
2. The model used.
3. The formula used by the model. User can modify the USER MODEL, the others are not modifiable.
4. The back-calculated fish length.
5. The slope of the allometric relation.
6. The intercept of the allometric relation.

You can use the TNPC integrated models or create your own ones using 2 and 3 . The existing models are :

- The Dahl-Lea model : Li = (Si * Lc)/Sc .
- The regression model : Li = a * Si + b .
- The SPH model (Scale Proportional Hypothesis) : Li = (((Lc - b) * Si) / (a * Sc) - b) / a .
- The BPH model (Body Proportional Hypothesis) : Li = (((Lc - b) * Si) / (a * Sc) - b) / a .

The variables used are :

- Li : back-calculated length of the fish.
- Si : length between the marker and the radial origin.

The constants are :

- Lc : fish's length when captured.
- Sc : radial length.
- a : allometric relation slope.

- b : allometric relation intercept.

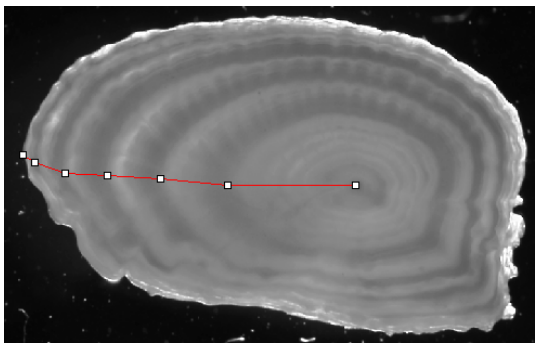
To build a new model, select a USER MODEL and modify the equation using the variables and constants available and the standard operators.

4.3. Position radials and markers

4.3.1. Position radials

To create a radial, click on . Right click on the center of the otolith (the center of the circles). On each right click, you will end the segment and start the new one. Continue until the end of the radial where you must double-click to end the segment creation. Click again on to close the radial creation.

Figure 4.11. Simple radial

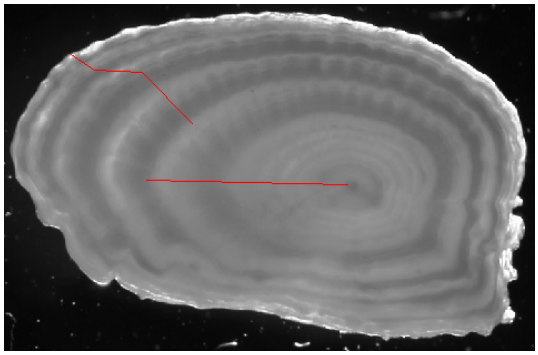


Note

The segmented lines are automatically rescaled to the CS edge. If you want not to, you will need to change the Find edge property in the settings. See *Section 4.2, "Settings"* for more information.

If the analysis cannot be realised along a simple trajectory, you can draw several trajectories. They will be linked according to their creation order.

Figure 4.12. Two parts radial



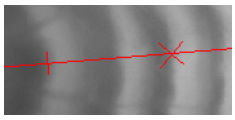
Note

The treatment steps defined in the settings are automatically applied after the radial creation. See *Section 4.2, "Settings"* for more information on treatment.

4.3.2. Position markers

To help interpretation of CS, 2 types of markers can be used : GR markers and CH markers. To add GR markers, click on . To add CH markers, click on . You then just have to click on the radial, where you want to place the marker. To remove a marker, just double click on it when its type is selected.

Figure 4.13. GR marker - CH marker



4.3.3. Open/Save radials

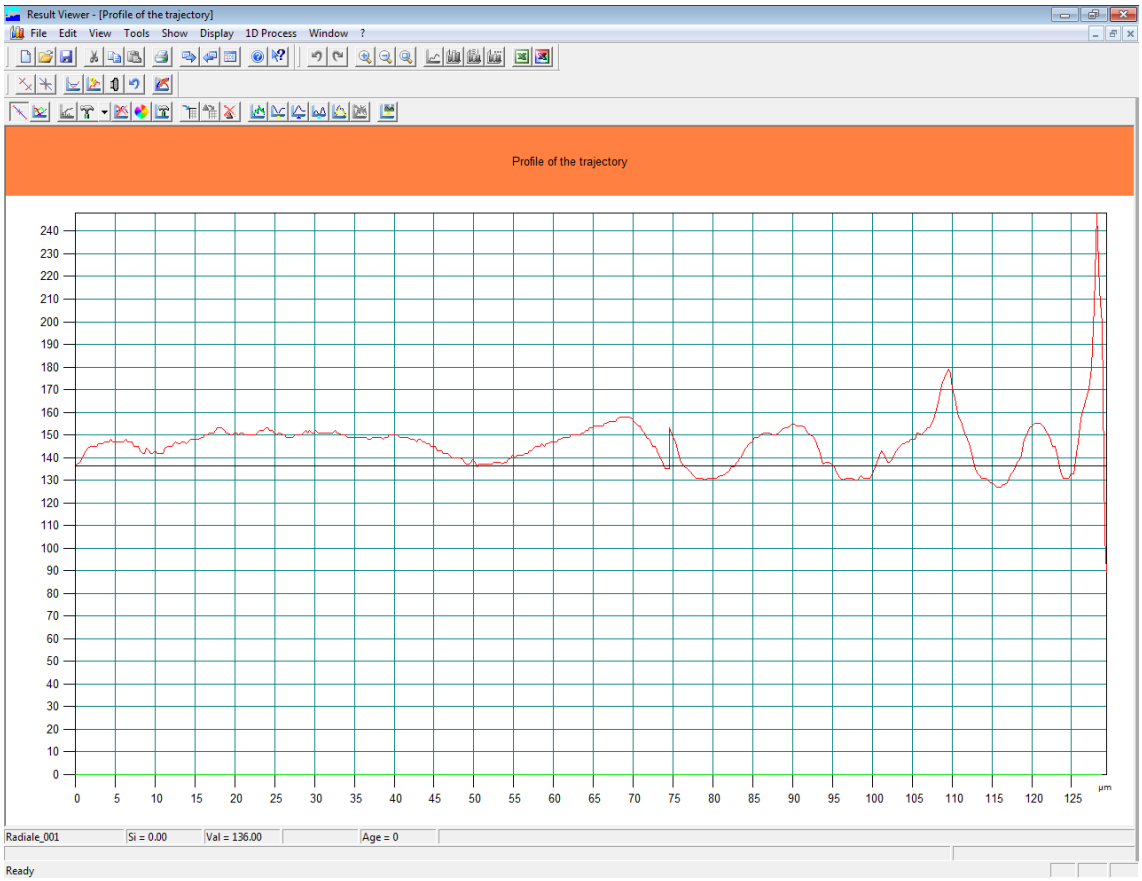
When you have finished analyzing a radial, you can validate it by clicking on . This will realise export and save operations. If you are using a database as source, it will also load the next image.

If you want to analyse an image that have already been analysed, you can click on . This will load the stored radial and markers.

4.4. Graphical results

The intensity profile and the growth measures linked can be visualized and analysed in the RESULT VIEWER. Use the RESULT VIEWER toolbar to analyse interactively with the intensity profile (markers positioning, automatic age calculation, interpolation…).

Figure 4.14. The Result Viewer with an intensity profile



4.4.1. Intensity profile

RESULT VIEWER shows the intensity profile of the selected radial if the option is selected (see *Section 4.2, "Settings"*). You can perform different actions using the TNPC toolbar.

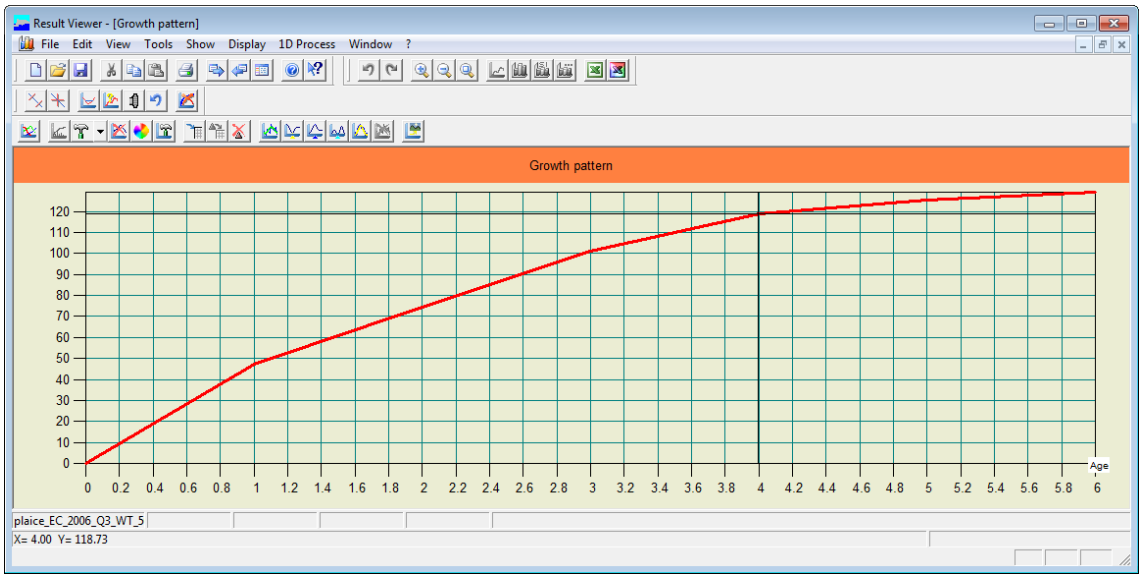
Figure 4.15. TNPC toolbar in Result Viewer

1. Add GR markers (see *Section 4.3.2, "Position markers"*).
2. Add CH markers (see *Section 4.3.2, "Position markers"*).
3. Put to 0 parts of the profile.
4. Interpolate markers between two points of the profile.
5. Do the selected processes (see *Section 4.2.3, "Process (Expert Mode)"*).
6. Undo the selected processes.
7. Erase the growth pattern chart.

4.4.2. Growth pattern

The RESULT VIEWER can also show the growth pattern associated to the current image interpretation. It can help the user to evaluate its interpretation's pertinence. The growth pattern can be erased using

Figure 4.16. Growth pattern in Result Viewer



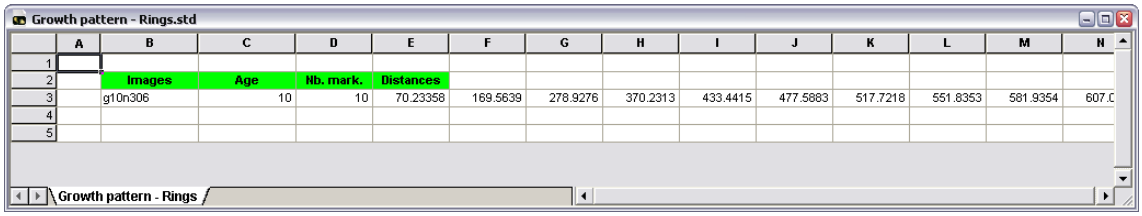
4.5. Numerical results

The RESULT VIEWER can also be used to build tables containing results about the growth and age, and more precisely :

- the age,
- the otolith's growth pattern,
- the position of the false rings or checks,
- the back-calculated fish length (if the option is activated).

The calculations on the current image are added to those from the previous images of the session. Once all the images have been analysed, the table can be exported to Microsoft® Excel™. Those data can also be stored in the database. This option must be activated in the settings (See *Section 4.2, "Settings"*).

Figure 4.17. Numerical results in Result Viewer

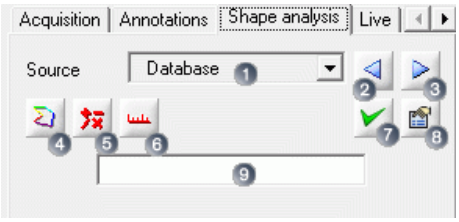


Chapter 5. Shape analysis (Expert Mode)

5.1. Generalities

The SHAPE ANALYSIS section allows the user to make measurements on the representative shapes of the observed CS. Automated functions can extract edges and calculate interesting values for the shape but you can use the length measurement toolbar for manual processing. You can perform the shape analysis on the current image or successively to the database images.

Figure 5.1. Shape analysis tab

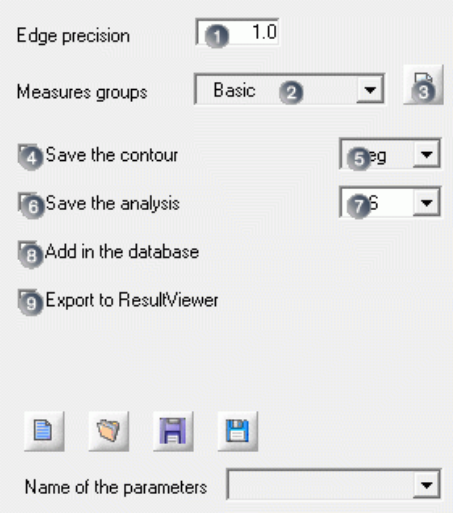


1. The image source
2. Previous image (only when using database source)
3. Next image (only when using database source)
4. Edge extraction
5. Otolith analysis. This will perform all the measures selected (See *Section 5.2, "Settings"*).
6. Show/hide the measures toolbar.
7. Validate analysis for this image, go to next image when using database source
8. Settings
9. Name used for data export. By default, this is the image name.

5.2. Settings

The settings rules the shape analysis as it is there you can change the analysis that is made on the shape, if the analysis is stored, added in the database…

Figure 5.2. Shape analysis settings



1. The edge precision
2. The group of measures to perform
3. Modify measures groups (cf *Section 5.2.1, "The measures groups"*)
4. Save the contour in a file located in the `Msr` folder.
5. The contour file extension, either `seg` or `dat`. `seg` is a Noesis file format. It contains information on the image and the associated data (size, type, raw data). `dat` is a text format.
6. Save the analysis in a file located in `Msr` folder.
7. The analysis file extension, either `a6` or `dat`. `a6` is a Noesis file format. It contains information on the number and the coordinates of the segments. `dat` is a text format.
8. Add the contour and the analysis in the database.
9. Export the results of the analysis in the RESULT VIEWER performed.

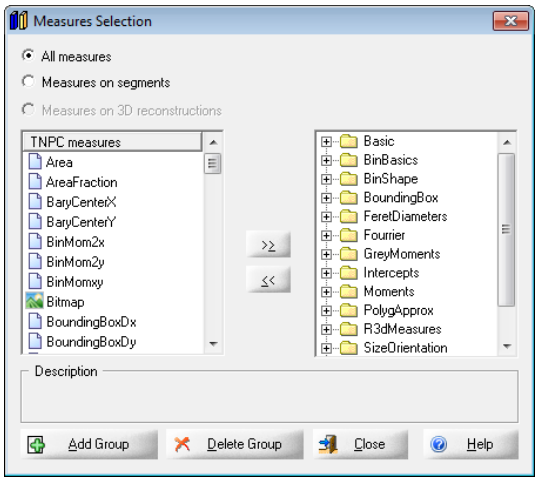
You can show or hide the settings by clicking on .

5.2.1. The measures groups

The measures that are performed are defined in the settings. You can chose the selected group using the list. By default, the selected group is BASICS.

If you want to know what measures will be performed by which group, or modify a group, click on . That will open the MEASURES SELECTION window.

Figure 5.3. Measures Selection



On the left part, you got all the available measurements. On the right, you got a tree representing each group and the measures in each group. To add a measure to a group, select the group in the right part and the measure in the left part and click on >>. To remove a measure from a group, select it in the right part and click on <<. You can also add or delete groups.

5.2.2. Other settings

In addition to the measure group settings, you have settings to save the contour and analysis or not, export those values to the RESULT VIEWER and/or the database. You can also change the precision of the edge detection algorithm. Like in all the tabs, you can save those settings.

5.3. Results

5.3.1. Edge extraction

To obtain information about otoliths' shape, you have to extract the otolith edge first. To access otolith's edge extraction, you have to click on . This function extract the background by applying thresholds on the picture before calculating a polygonal approximation of the edge. This approximation uses precision parameters specified in the settings (see *Section 5.2, "Settings"* for more information).

To perform a correct edge detection, you have to adjust the thresholds in the grey levels bar under the picture. Make sure all but only the otolith is in red (some noise might still be in red, it will be discarded by the polygonal approximation).

Figure 5.4. Good threshold - Bad threshold

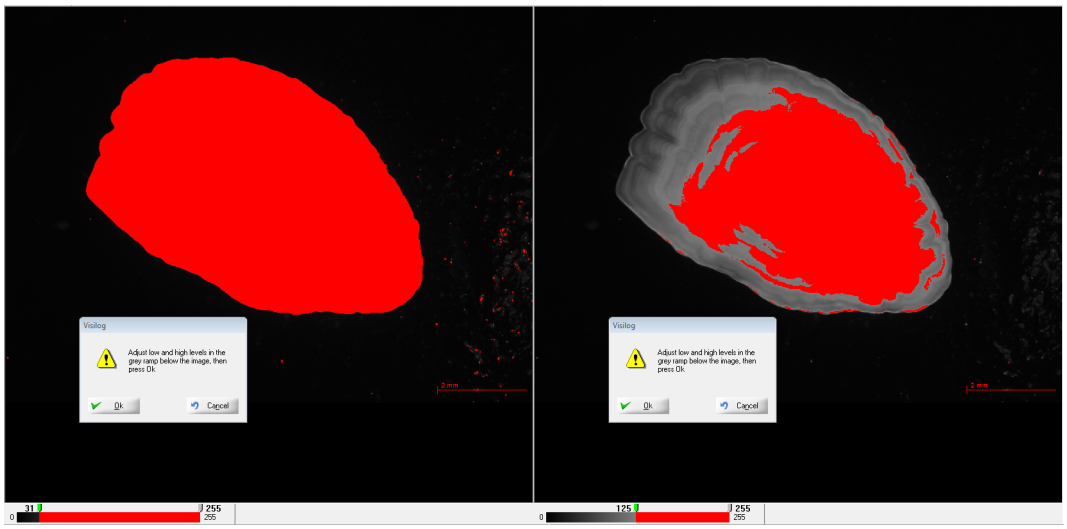
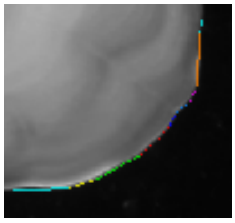


Figure 5.5. Edge detection result

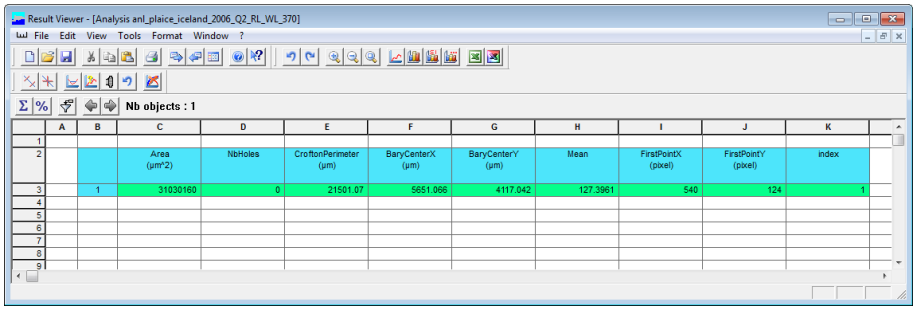


5.3.2. Otolith analysis

Several parameters can be calculated from the otolith and stored (perimeter, surface, Fourier moments… see *Section 5.2, "Settings"* for more information).

To perform the otolith analysis, click on . If you have not done the edge extraction before, it will automatically be performed at this time (see *Section 5.3.1, "Edge extraction"* for more information). The calculation is run and then you got the result in the RESULT VIEWER.

Figure 5.6. Analysis result

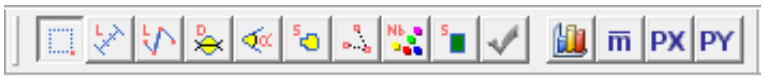


You can then either quit the RESULT VIEWER and validate your analysis or continue analysing the image.

5.3.3. Measurements

The MEASURE BAR features all the tools needed to manually measure everything in an image. To display this toolbar, you can click on VIEW → MEASURE BAR or on . We will not explain the buttons as they are in the Visilog 6™ manual. Please refer to it for more information.

Figure 5.7. Measure bar



Chapter 6. Live (Expert Mode)

6.1. Generalities

The LIVE mode allows you to acquire and annotates images at the same time. If the zone of interest is bigger than the image size, TNPC will automatically create a mosaic image.

Figure 6.1. Live tab

1. Create radial
2. Add GR markers
3. Add CH markers
4. Open radial
5. Delete all radials and markers
6. Change radials and markers colors
7. Perform interpolation
8. Validate the radials and markers positioning and launch the mosaic creation if needed. The information stored are configured in settings.
9. Open settings.
10. Start image acquisition.
11. Name used for the different file saved : image, radial, results…

6.2. Settings

To display the settings tabs, you just have to click on .

6.2.1. General

The general settings allows you to parametrize the name and number of the image acquired.

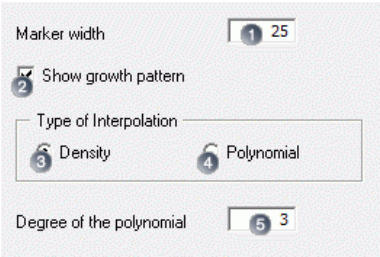
Figure 6.2. General settings

1. Generic name used for the saves (image, radials, results…). All the files will be named by this name followed by a number.
2. Number of the next otolith that will be stored. All the files saved (image, radials, results…) will use this number.

6.2.2. Checks

The checks settings allow you to manage the interpolation function (cf *Section 4.2.4, "Interpolation (Expert Mode)"*) and the markers size configuration.

Figure 6.3. Checks settings

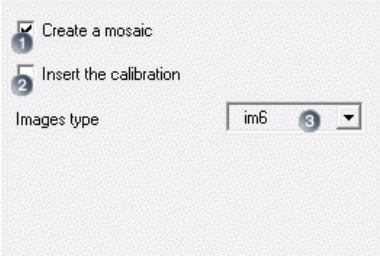


1. You can set here the marker size.
2. Show the growth pattern in the RESULT VIEWER after calculation.
3. Activate the density interpolation.
4. Activate the polynomial interpolation.
5. The degree of the polynomial interpolation.

6.2.3. Acquisition

The Acquisition settings allows you to choose the image type of the image acquired, and some parameters about the acquisition.

Figure 6.4. Acquisition settings

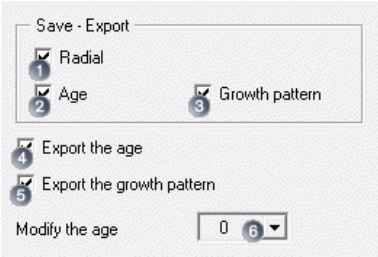


1. Allow the automatic creation of mosaic pictures.
2. Insert the calibration in the picture (might be a mosaic).
3. Choose the output format of the image. 5 formats are available. For more info, read *Image format* .

6.2.4. Export

In this tab you can manage all the export settings.

Figure 6.5. Export settings



1. Store the radial in a `.rad` file located in the `/rad` folder.
2. Store the estimated age.
3. Store the growth pattern in a `.iid` file located in the `/iid` folder.
4. Store the estimated age in a datasheet in the graphic window.
5. Store the growth pattern in a datasheet in the graphic window.
6. Add or remove one to the number of rings for age calculation.

6.2.5. Back-calculation

This tab is the exact same than for annotation. Please refer to *Section 4.2.5, "Back-calculation"* for more info.

6.3. Otolith live annotation

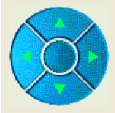
6.3.1. Start image acquisition

To start the acquisition, click on . The image coming from the camera will be displayed on the screen. You can then start the creation of the radial and add rings or false rings to it.

6.3.2. Create radials

To start creating a radial, click on . You will see an icon enabling the motorized stage movements appear.

Figure 6.6. Motorized stage movements icon



Warning

Always use the motorized stage movements icon to move the stage. If you use your controller joystick, the radial creation will fail.

You can effectively start the radial creation by clicking on the image on the start point. You can then select intermediate points by clicking on their location on the image. If you need to move the stage, it is advised to position a radial point near the image edge by double clicking on the it, that will pause the radial drawing. You can then move the motorized stage using the icon (left, right, up or down). Once the radial is at the desired position, you can continue the radial drawing by clicking on the image at the desired point.

Note

You can specify the movement step by right-clicking on the motorized stage movement icon arrows. For an ease of use, it is advised to define the step size to 2/3 of the field.

6.3.3. Position markers

You can add GR and CH markers to the radial. Click on to add GR markers or on to add CH markers and then click on the image on the marker position. To remove a marker, simply double-click on it.

6.3.4. Interpolation

Activate the interpolation by clicking on . Choose the zone of interest by placing two markers at the beginning and at the end of the zone and click OK on the pop-up window. A new window should pop up, just slide the two markers at the start and end of the zone where new markers should be computed. Just validate the choice by clicking on OK in the window.

6.3.5. Validation and exportation

Once you have drawn your radial and positioned your markers, you can validate and store the image by clicking on . This will create the mosaic needed, perform all the processes you set up and store the image, radials, results… according to the settings.

Chapter 7. Automatic estimation (Expert mode)

The automatic estimation is a process that needs an opened database. You first need to open the database you want to work on.

Note

For the process to be correct, you will have to estimate by hand a few otoliths. The more otoliths you estimate by hand, more precise the estimation will be.

Note

If you want to know more about automatic ageing, if you want to change the default values, please refer to the *AFISA (Automatic FISh Ageing) publications* [http://ec.europa.eu/research/fp6/ssp/afisa_en.htm] .

7.1. Apprenticeship of individual age

7.1.1. Generalities

To perform an automatic estimation of age, you will need to have a codebook. Either a codebook is present for the species you are studying, and you will not need to perform this step, or you might want TNPC to learn how to estimate age through the estimation you already made.

To set up the apprenticeship of individual age, select the APPRENTICESHIP OF INDIVIDUAL AGE tab.

7.1.2. Settings

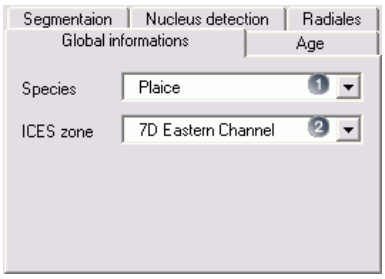
Before launching the apprenticeship, you have to fill in some required settings.

7.1.2.1. Global information

The first thing to do is to select the species you want to study and its ICES [1] zone.

1 International Council for the Exploration of the Sea

Figure 7.1. Apprenticeship of individual age - Global information

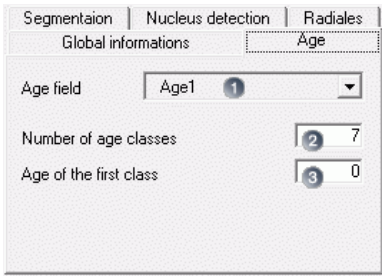


1. Select here the species you are studying.
2. Select here the ICES zone of the species you are studying.

7.1.2.2. Age

In this tab, you have to select the field in database where you estimated the age, the number of age classes and the age of the first class.

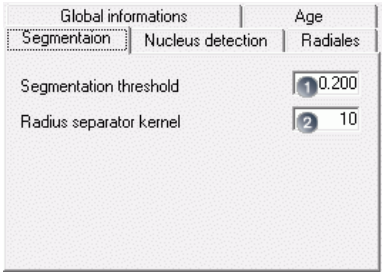
Figure 7.2. Apprenticeship of individual age - Age



1. Select here the field where you estimated the age in.
2. Insert here the number of different age class.
3. Insert here the age of the first class. All the ages before the first class will be identified as being of the first class; For the last class, it works the opposite way.

7.1.2.3. Segmentation

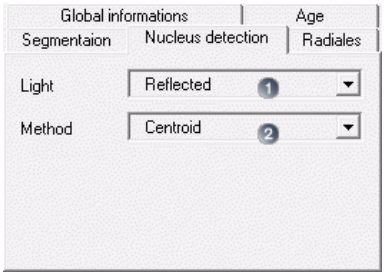
Figure 7.3. Apprenticeship of individual age - Segmentation



1. Insert here the threshold used for segmentation. You should not need to change the default value.
2. Insert here the radius separator kernel. You should not need to change the default value.

7.1.2.4. Nucleus detection

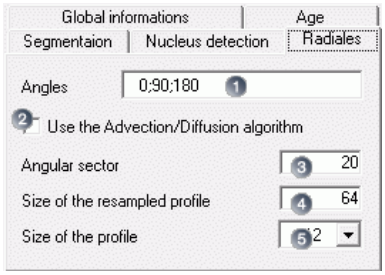
Figure 7.4. Apprenticeship of individual age - Nucleus detection



1. Select here the type of light of your hardware between REFLECTED and TRANSMITTED.
2. Insert here the method used for nucleus detection between CENTROID, MORPHOLOGICAL or EMPIRIC.

7.1.2.5. Radials

Figure 7.5. Apprenticeship of individual age - Radials

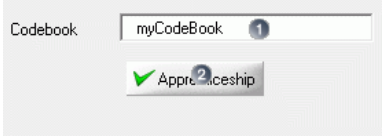


1. Insert here the angles of the radials that will be created for the automatic estimation. Those angles must be separated by ';'. The more angles, the longer the apprenticeship will be and the estimation after.
2. Select if you to use the Advection/Diffusion algorithm. This algorithm cleans the image.
3. Angular sector around the radial.
4. The size of the resampled profile. The bigger the size is, the longer the generation, and later the estimation, will be.
5. The size of the profile. The bigger the size is, the longer the generation, and the estimation, will be.

7.1.3. Create the learning file

To create the learning file, enter its name and launch the apprenticeship.

Figure 7.6. Create the learning file



1. Insert here the name of the generated learning file (the codebook). This codebook will be available for this species in this ICES zone.
2. Launch the codebook generation.

The learning file, the codebook, will be stored in `data/Tnpc/CodeBook` in your TNPC installation directory.

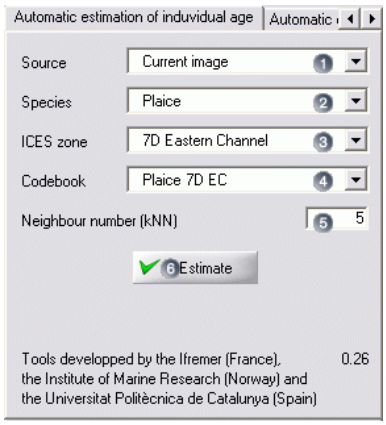
This text file contains the parameters defined and the matrix used to auto estimate the age. You can generate this file by hand without using TNPC (but SciLab™, MatLab® or other mathematical software).

7.2. Automatic estimation of individual age

7.2.1. Launching the estimation

To launch the estimation, you will need to select the AUTOMATIC ESTIMATION OF INDIVIDUAL AGE. Then, you will have to fill in some parameters such as the source of data, the species, ICES zone and codebook to use and then launch the estimation clicking on Estimate.

Figure 7.7. Automatic estimation of individual age

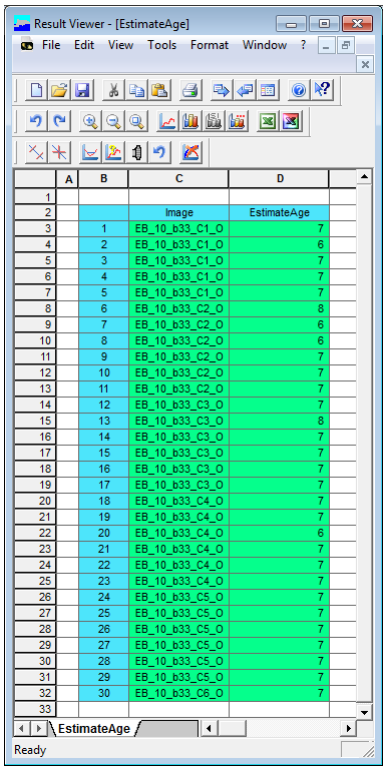


1. Select here the source. Either the current image if you want to estimate a single image, or the database if you want to estimate all your database.
2. Select here the species you are studying.
3. Select here the ICES zone of your species.
4. Select here the codebook to use. You can use predetermined codebooks or the ones you created.
5. The Neighbor number parameter in the Nearest-neighbor interpolation.
6. Launch the estimation.

7.2.2. Results

The estimation might take some time to complete. Once it is finished, you have the results in the RESULT VIEWER. You can also find the results in the database in the field AUTOESTIMAGE.

Figure 7.8. Automatic estimation of individual age



	A	B	C	D
1				
2			Image	EstimateAge
3		1	EB_10_b33_C1_O	7
4		2	EB_10_b33_C1_O	6
5		3	EB_10_b33_C1_O	7
6		4	EB_10_b33_C1_O	7
7		5	EB_10_b33_C1_O	7
8		6	EB_10_b33_C2_O	8
9		7	EB_10_b33_C2_O	6
10		8	EB_10_b33_C2_O	6
11		9	EB_10_b33_C2_O	7
12		10	EB_10_b33_C2_O	7
13		11	EB_10_b33_C2_O	7
14		12	EB_10_b33_C3_O	7
15		13	EB_10_b33_C3_O	8
16		14	EB_10_b33_C3_O	7
17		15	EB_10_b33_C3_O	7
18		16	EB_10_b33_C3_O	7
19		17	EB_10_b33_C3_O	7
20		18	EB_10_b33_C4_O	7
21		19	EB_10_b33_C4_O	7
22		20	EB_10_b33_C4_O	6
23		21	EB_10_b33_C4_O	7
24		22	EB_10_b33_C4_O	7
25		23	EB_10_b33_C4_O	7
26		24	EB_10_b33_C5_O	7
27		25	EB_10_b33_C5_O	7
28		26	EB_10_b33_C5_O	7
29		27	EB_10_b33_C5_O	7
30		28	EB_10_b33_C5_O	7
31		29	EB_10_b33_C5_O	7
32		30	EB_10_b33_C6_O	7
33				

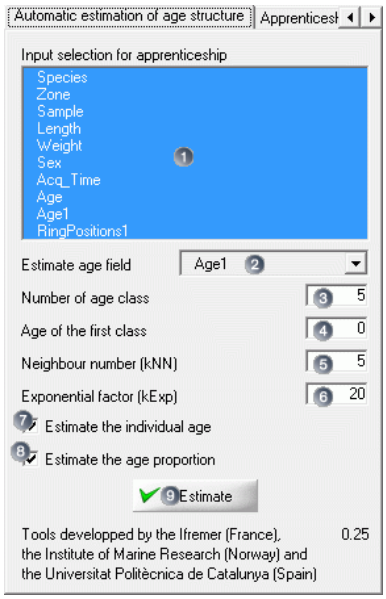
7.3. Automatic estimation of age structure

7.3.1. Launch the estimation

The automatic estimation of age structure consist in estimating quickly all the ages, already estimated or not, and adjusting the model using the already estimated ages. The algorithm used for this estimation is the Nearest-neighbor interpolation.

To perform the automatic estimation of age structure, open the AUTOMATIC ESTIMATION OF AGE STRUCTURE tab.

Figure 7.9. Automatic estimation of age structure window



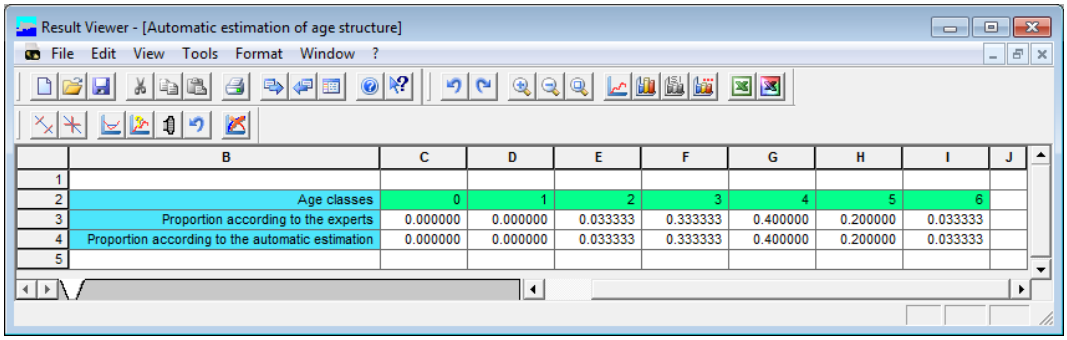
1. Select here the fields of the database you want to use for the apprenticeship. An estimated age field must be in this selection.
2. Select here the field where the age you estimated is present.
3. Insert here the number of different age class.
4. Insert here the age of the first class. All the ages before the first class will be identified as being of the first class; For the last class, it works the opposite way.
5. Parameter of the Nearest-neighbor interpolation.
6. Parameter of the Nearest-neighbor interpolation.
7. Check this box to estimate the individual age.
8. Check this box to estimate the age proportion.
9. Launch the estimation.

Once you have selected all the parameters, you can launch the estimation by clicking on Estimate.

7.3.2. Results and export

The results of the estimation are showed in the RESULT VIEWER. You can also find the estimation log in the file named `estimateAgeProp.log`. In this file, you will see the input parameters as well as the results.

Figure 7.10. Automatic estimation of age structure estimation results



	B	C	D	E	F	G	H	I	J
1									
2	Age classes	0	1	2	3	4	5	6	
3	Proportion according to the experts	0.000000	0.000000	0.033333	0.333333	0.400000	0.200000	0.033333	
4	Proportion according to the automatic estimation	0.000000	0.000000	0.033333	0.333333	0.400000	0.200000	0.033333	
5									

You have also the estimated age in the database on the column `AutoEstimAge_2`.

Chapter 8. Extend TNPC

8.1. Translate TNPC in your language

If you want to use TNPC in your language, but it is not present in the TNPC installation, you can translate TNPC by yourself. To do so, copy the directory `data/Tnpc/lang/en` and rename it, for example in `es` if you want to translate TNPC in Spanish.

In this copied directory, open all the `.lng` files, and translate all the sentences. Each sentence is displayed like in *Example 8.1, "Sentence to translate"* . The first part is a key, the second part is the type of message, the third part is the displayed sentence. This is this third part that you need to translate.

Example 8.1. Sentence to translate

```
"frmAcqMosaique","Caption","Acquisition -- Mosaic"
```

Example 8.2. Sentence translated in Spanish

```
"frmAcqMosaique","Caption","Adquisición -- Mosaico"
```

Once all your sentences are translated, you have to change the language in the file `data/Tnpc/AddLang.cfg`. You can see the content of this file in *Example 8.3, "The `AddLang.cfg` file"* . Between the empty quotes, put you language code. For example, for Spanish put `es`.

Tip

The language code correspond to the name of your translated files directory. You can name it with more explicit names if you prefer, but will need to report this name in this file.

Example 8.3. The `AddLang.cfg` file

```
"Additional Language",""
```

8.2. Add/Change species available

If some species you want to study are not available in TNPC, or if you only use some of the provided species, you might want to add the missing species and remove the useless ones. To do so, open the file `data/Tnpc/lang/yourLanguage/Species.cfg`. After the first line stating the version of TNPC, you have the list of species, with one species per line. Each line is compounded of two values between quotes. The first one is the scientific name, the second one is the common name. You can add new species at the end of the file or remove the lines corresponding to the useless species.

Example 8.4. The `Species.cfg` file

```
"Version",500
"","Grey gurnard"
```

```
"","Long finned gurnard"
"","Tub gurnard"
"Aspitrigla cuculus","Red gurnard"
"Clupea harengus","Atlantic herring"
```

Tip

Before removing species, you might want to backup this file, for example copying it under the name `Species.cfg.old`.

8.3. Change the available species for Automatic Estimation

By default, in the automatic estimation, only Cod and Plaice are available and for only two ICES zone each. You might want to add species and ICES zones. To do so, open the file `data/Tnpc/lang/yourLanguage/Aea_Species.cfg`. This file contains, for each species, the corresponding ICES zone, and the prefix used for the default codebook name. You just need to adapt this file to your needs, adding species or ICES zone for a species by adding lines to this file, or changing the prefix used.

Example 8.5. The `Aea_Species.cfg` file

```
"Version",500
"Cod","4 North Sea","NScod"
"Cod","2A North East Arctic","NEAcod"
"Plaice","7D Eastern Channel","ECplaice"
"Plaice","5A Iceland","ICplaice"
```

Note

The prefix will be followed by `_R_Codebook`, for example NScod_R_Codebook.

8.4. Add your own codebooks

You can add your own codebooks by adding them in `data/Tnpc/CodeBook`. They must be in the `.cbk` format.

Chapter 9. Export files

Several files can be created by TNPC :

- The radials files got the `.rad` extension. They store the appearance, position and length of the radials. They can be loaded by TNPC in order to be read again.
- The growth measures are stored in `.iid` files. They are CSV files that cannot be read by TNPC but by any text editor.
- The `.pro` files store the intensity profile value. They are CSV files that cannot be read by TNPC but by any text editor.
- The `.dat` files store the shape analysis results or the otolith edge. They are CSV files that cannot be read by TNPC but by any text editor.
- The rings positions are stored in `.xyd` files. They are CSV files that cannot be read by TNPC but by any text editor.

9.1. Radials (`.rad` files)

Radials are saved in `.rad` files in a `/rad` directory. They can be loaded using the  button from the ANNOTATION tab. To save a radial, select the RADIAL checkbox from the EXPORT tab on the ANNOTATION parameters (see *Section 4.2.2, "Export"*). The save is done when the radial is created and validated by  . By default, the file created has the same name than the image (except for the extension).

9.2. Markers (`.iid` files)

The distances between the markers can be saved. The files created are CSV files that got the same name than the images (by default) and the `.iid` extension (for Inter Incremental Distance). They are saved in the `/iid` directory and are coded the following way :

Example 9.1. `.iid` file content

```
Image name (1)
Xn;Yn (2)
Xed;Yed (3)
Xs;Ys (4)
Xen;Yen (5)
XL0;YL0 (6)
Calibration (7);RadImaRatio (8)
Species;Catch zone (9)
Catch date;Sample (10)
```

```
Length;Weight;Sex (11)
Nb (12);Distance (13);Back-calculated distance (14)
Edge;Distance (15)
```

1. The image name
2. The nucleus coordinates
3. The edge coordinates at the profile level
4. The profile start coordinates
5. The profile end coordinates
6. The L0 point coordinates
7. The pixel size
8. Scale ratio between the image and the profile
9. Parameters entered by the user from the database
10. Parameters entered by the user from the database
11. Parameters entered by the user from the database
12. Marker's number
13. Marker's distance from the nucleus
14. Back-calculated distance from the otolith center if the Length when captured have been given.
15. Edge's distance from the nucleus

9.3. Profiles (`.pro` files)

TNPC stores the grey levels of the pixels located on the radial and the markers position on a `.pro` file. They are CSV files located in the `/pro` directory and are coded the following way :

Example 9.2. `.pro` file content

```
Image name (1)
Xn;Yn (2)
Xed;Yed (3)
Xs;Ys (4)
Xen;Yen (5)
XL0;YL0 (6)
Calibration;RadImaRatio (7)
Species;Catch zone (8)
Catch date;Sample (9)
Length;Weight;Sex (10)
PixelsNb (11)
```

```
Pixel(12); Distance1(13); Distance2 (14); Check (15)
```

1. The image name
2. The nucleus coordinates
3. The edge coordinates at the profile level
4. The profile start coordinates
5. The profile end coordinates
6. The L0 point coordinates
7. The pixel size and the scale ratio between the image and the profile
8. Parameters entered by the user from the database.
9. Parameters entered by the user from the database.
10. Parameters entered by the user from the database.
11. Number of pixels in the profile.
12. Grey level value of the pixel (between 0 and 255).
13. Distance from nucleus to the pixel
14. Anamorphosed distance from nucleus to the pixel
15. Presence or not of a marker (0 or 1)

9.4. Rings positions (.xyd files)

TNPC stores the rings positions values in `xyd` files.Those files contain the w and y position of the ring in the image and the distance from the radial start.

Example 9.3. .xyd file content

```
Version 500 (1)
Nb position 6 (2)
Ring(3) 323(4) 226(5) 41.679924(6)
Ring 411 202 99.899498
Ring 494 181 154.811142
Ring 607 238 214.195801
CheckRing(7) 651 241 242.424835
Ring 676 243 258.464050
```

1. File format version
2. The number of rings
3. The type of ring, here a real ring
4. The X coordinate
5. The Y coordinate

6. The distance from the radial start to the ring
7. A check ring

9.5. Shape Analysis (`.dat` files)

TNPC stores the shape analysis values in `dat` files. You can export either the edge or the measures. Those files contains the selected measures with, for each measure, their name and value.

Example 9.4. Edge `.dat` file content

```
X      Y      Index  Chain  Ext chain  Direction  Label   index
404.5  634.5  0      0      --1        1          Label1  1
387.5  631.5  1      0      --1        1          Label1  2
372.5  626.5  2      0      --1        1          Label1  3
372.5  622.0  3      0      --1        1          Label1  4
367.5  618.5  4      0      --1        1          Label1  5
364.5  613.0  5      0      --1        1          Label1  6
355.5  612.5  6      0      --1        1          Label1  7
351.5  606.0  7      0      --1        1          Label1  8
```

Example 9.5. Measures `.dat` file content

```
Area      NbHoles  CroftonPerimeter BaryCenterX BaryCenterY
196088.0  1        2244.36890       622.66083   462.03506
```

Appendix A. List of cameras supported by TNPC

This appendice provide a list of camera supported by TNPC. It was last updated the 9th march 2011. Some new camera might be supported since then. If your camera works with TNPC and is not present in this list, please tell us so that we can update this list.

A.1. IEEE1394 Cameras

Cameras that respect the DCAM/IIDC norm are well supported (like Sony, Hamamatsu, Baumer and JVC).

Cameras not respecting those norms (like Leica, Nikon, Olympus or Zeiss) are not supported.

A.1.1. Sony

- SONY CCM-DS250
- SONY DFW-V300
- SONY DFW-V500
- SONY DFW-VL500
- SONY XCD-X700
- SONY XCD-SX900
- SONY DFW-X700
- SONY DFW-SX900
- SONY XCD-X710
- SONY XCD-X710CR
- SONY XCD-SX910
- SONY XCD-SX910CR
- SONY DFW-X710
- SONY DFW-SX910
- SONY XCD-V50
- SONY XCD-V50CR

- SONY XCD-U100CR

A.1.2. JVC

- JVC KY-F1030
- JVC KY-F75

A.1.3. SPOT

- SPOT FLEX
- SPOT Insight5
- SPOT RT3

A.1.4. Hamamatsu

- Hamamatsu C4742
- Hamamatsu C8100
- Hamamatsu C8484
- Hamamatsu C9260
- Hamamatsu C9440
- Hamamatsu ARGUS20
- Hamamatsu C10600

A.1.5. Baumer Optronic

- Baumer Optronic DWX
- Baumer Optronic TXD

A.2. USB Cameras

All the cameras with a Microsoft Windows driver compatible with WDM are supported by TNPC.

Amongst the other cameras, only the Lumenera cameras are supported.

A.2.1. Lumenera

- Lumenera Infinity

A.3. GigEthernet Cameras

Only Baumer cameras are supported.

Index

Glossary

This glossary explains all the important notions involved in TNPC.

A

AFISA

AFISA stands for Automatic FISh Ageing. This is a European research project aimed to develop standardised systems to automatically age fish stocks. Those systems should reduce fish ageing costs and harmonized methods between the different European laboratories.

You can see the *AFISA website* [http://ec.europa.eu/research/fp/ssp/afisa-en.htm] for more information.

Allometry

Allometry is the study of the relationship between size and shape. It is a well-known study, particularly in statistical shape analysis for its theoretical developments, as well as in biology for practical applications to the differential growth rates of the parts of a living organism's body. The relationship between the two measured quantities is often expressed as a power-law such as : $W = a.L^b$. With "W" the weight of the animal body, "L" the length of the animal body, "a" the intercept and "b" the slope (growth coefficient of fish relative growth rate).

B

Back-calculation

The derivation of growth history of individuals from measurements of increments within otoliths (or calcified structures). Correct application requires a model of the relationship between otoliths (or calcified structures) growth and fish somatic growth and assumptions about the constancy of increment deposition. You can refer to Green, BS and Mapstone, BD and Carlos, G and Begg, GA, Tropical Fish Otoliths: Information for assessment, management and ecology, Springer, Dordrecht, pp 313. ISBN 978-1-4020-3582-1 (2009) for more information.

C

Calcified Structure (CS)

A Calcified Structure or Calcified Piece is a part of living being made mostly of calcium carbonate, such as coral reefs, shellfishes, or fishes otoliths. It can usually be studied using sclerochronology.
See Also *Sclerochronology*, *Otolith*.

Check marker (CH marker)
: A check (or CH) marker is used to identify false rings, checks… on a radial.
See Also *Radial*.

Conseil International pour l'Exploitation de la Mer (CIEM)
: See *International Council for the Exploration of the Sea* .

G

Growth structure marker (GR marker)
: A GR marker is used to identify growth structures on a radial.
See Also *Radial*.

H

Halieutics
: Halieutics can be defined as "The science of living aquatic resources running". It tends to integrate new dimensions such as resource management or restoration, in a sustainable development way.

I

International Council for the Exploration of the Sea (ICES)
: ICES is the oldest intergovernmental organisation in the world concerned with marine and fisheries science. It coordinates and promotes marine research on oceanography, the marine environment, the marine ecosystem, and on living marine resources in the North Atlantic.

  You can refer to the *ICES/CIEM website* [http://www.ices.dk/indexfla.asp] for more information.

Image format
: An image format is the way the image is stored. Several formats exists, with their pour and cons. Five of them are available in TNPC.

  - im : Visilog ™ image file. Can be read only by people having Visilog™ or TNPC. Now using the `im6` file format with Visilog 6™.
  - tif : format allowing to store big bitmap images without loss. Tif format is platform independent (anyone can read it). You can add a loss-less compression (LZW) in TNPC preferences :
    TOOLS → SETTINGS → OUTPUT IMAGE FORMAT → TIFF → COMPRESSION
  - jpg : Format allowing to store image with compression and loss. Can typically achieves 10:1 compression with little perceptible loss in image quality. Platform independent format (can be read by anyone).

- ppm/pgm : Format of images stored in plain ASCII text. Files are stored without loss but can be bigger than with other formats. Platform independent format (can be read by anyone).

N

Nucleus

The nucleus indicates the core or primordia of the otolith. The nucleus is the formation center of the otolith, the point from which all the radials start.
See Also *Radial*, *Otolith*.

O

Otolith

An otolith is a calcified structure of the inner ear of fishes. This part grow with the fishes and growth rings appears on it, making it the perfect piece to study to determine fish age.
See Also *Sclerochronology*, *Calcified Structure*.

R

Radial

A radial is a line going from the nucleus of the otolith to the outer limit (it might be segmented). It is used to place markers in order to determine fishes age.
See Also *Growth structure marker*, *Check marker*, *Nucleus*.

Reader

Reading a calcified structure consist in interpreting its growth patterns. A reader tries to interpret the marks recorded in a given structure.

Result Viewer

The Result Viewer is a window allowing the user to see charts and tables and perform some calculations. To open it click on TOOLS → RESULT VIEWER.

Ring

The rings or bands are used for fish ageing. Generally, one ring is described by one light zone and one dark zone.

S

Sclerochronology

Sclerochronology is the science that studies living being by counting growth rings in their calcified structures (otoliths, scales, skeletal tissue…). Its aim is to determine the age and the period and length of important events on a specimen's life.

Stage

A stage is the part of a microscope where you put what want to study. It can be fix or motorized. In the case of motorized stages, you can usually move the stage on the X and Y axis and sometimes on the Z axis too.